RNA Biology
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Lecture - 60
Epitranscriptome and Protein Synthesis Biological Implications of RNA Modifications

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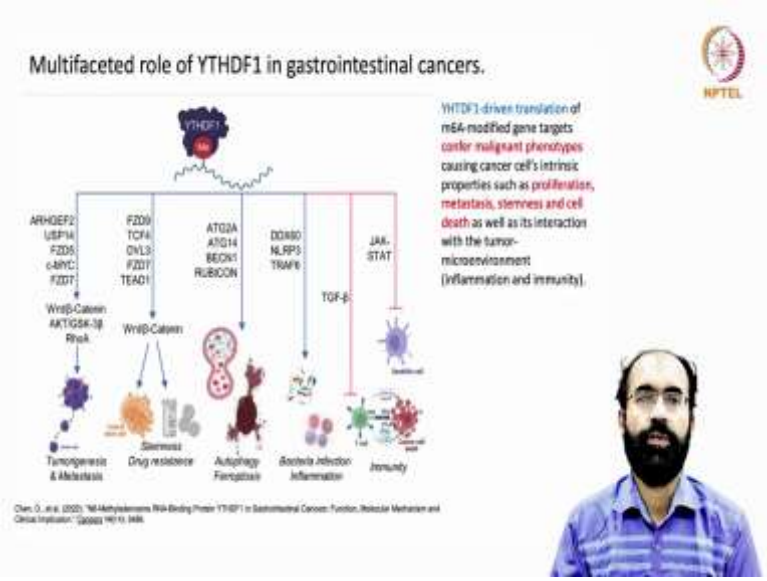

Hello everyone. Welcome back to another session of RNA Biology. So, we were studying the impact of post-transcriptional modifications that is occurring on the RNAs, especially the modification that is causing a methylation of the adenine residues. And that can make the RNA stable and more translatable, and often up regulating several signalling pathways in the cell.

And which can often cause the deleterious effect such as diseases, various types of cancers, etcetera. So, let us see what are the other possible changes that can brought in by the YTHDF1 group of proteins.

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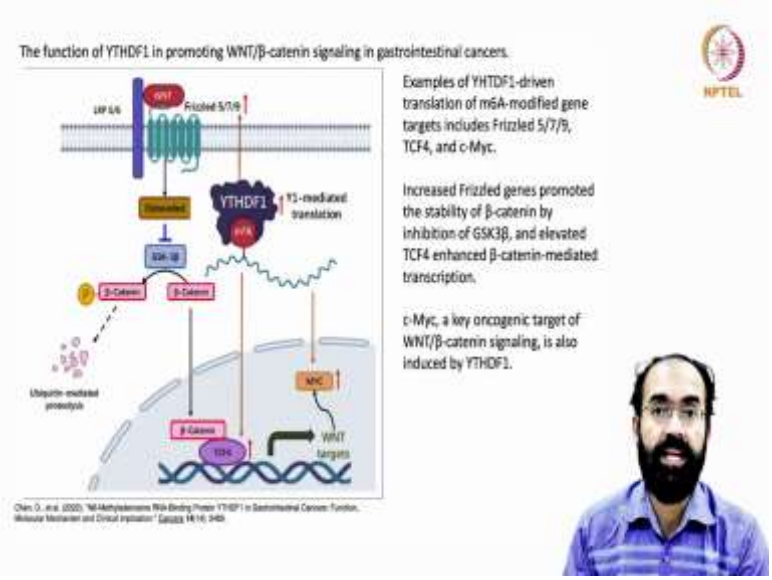

So, let us learn more in detail about how this reader that is YTH proteins basically are the readers that recognize the changes that is brought in to the adenine residues, especially the methylation change and that will mark the RNA for specific destination. Let us learn how it is done with regards to WNT beta-catenin signaling and leading to gastrointestinal cancers.

So, we can see here, I in the previous class mentioned verbally about the WNT signalling mechanism, the basics of the WNT signalling mechanism and let us see in a pictorial manner here. So, you have this receptor that is called frizzled, different types are there, frizzled 5, frizzled 7, frizzled 9 like that and it also have a core receptor LRP5-5 slash 6. So, LRP is the core receptor, frizzled is the main receptor. So, on to this main receptor, you have this ligand WNT that can bind.

So, it is originally derived from the drosophila that is the phenotype is wingless. So, that is the origin of WNT the name. So, what happens? Once the WNT is bound onto the frizzled, then what it can do? It can activate the protein dishevelled which is staying in the cytoplasm.

So, once it is activated, it can go and inhibit the GSK3 beta enzyme and this GSK3 beta enzyme is very important because it is important in the phosphorylation of the beta-catenin. If it is phosphorylated, the beta-catenin will be marked for degradation. So, the role of GSK3 beta is glycogen synthase kinase 3 beta that is the full name of GSK3 beta.

So, if the phosphorylation happens on the beta-catenin it will be degraded. Remember beta-catenin is an important molecule required for the housekeeping function, but the excess beta-catenin has to be marked for degradation without which it can find its way into the nucleus. So, once the dishevelled is activated it will prevent the GSK3 beta from phosphorylating it, hence preventing the degradation of the beta-catenin.

So, now, the beta-catenin is accumulated in the cytoplasm which will find its way into the nucleus nucleus beta-catenin along with a TCF factor. So, it is also known as T cell-factor. So, TCF is another protein. In collaboration with beta-catenin, it can turn on a bunch of genes known as WNT target genes.

Remember, this WNT target genes are invariably pro-mitotic genes; that means, they will push the cell into proliferation. Like I told you earlier, that any small injury happens in your body including your skin erosion or a small mosquito bite or a scratch wound, everything repairing require the WNT signaling. So, understand that the WNT signaling causes cell proliferation and which is meant for repairing function mainly.

So, one of the WNT target genes is the MYC. And what happens, this MYC often is considered a proto oncogene, which is important for the activation of various proliferative pathway.

So, now how the modification is coming into picture so, if you have the RNA of the WNT target gene in the nucleus, all the WNT target genes are made in the form of RNA and that will be pushed into the cytoplasm. And in the cytoplasm, it undergoes this modification m6A that is methylation of the adenine residue, which will be detected by the YTH domain containing protein.

So, this YTH will cause an accelerated translation of this WNT target genes and they will have more job to play than what usually it is. So, some examples of YTHDF1 driven translation of this m6A modified gene targets include the frizzled 5, 7, 9 like which are the members of the WNT signaling pathway TCF4 that is the T cell factor that is a co-protein or co-binding partner of the beta-catenin and also the c-Myc itself.

So, accelerated production of the frizzled will facilitate the WNT signaling because more receptor means whenever WNT is available none of the WNT will go wasted. And not

only that you have too much of TCF, so whatever stabilized beta-catenin you will find an efficient way of binding to the targets because there is abundant supply of TCF also now.

And not only that one of the targets that is the MYC, once it is produced more and more of the MYC RNA will be facilitated for translation. So, it simply makes sure that the purpose of WNT signaling will be super accelerated by activating the components of WNT signaling at different level, at receptor level, co-binding partner level and also the target gene level.

So, these things are very important and that can accelerate the proliferation mechanism. So, increased frizzled genes promoted the stability of beta-catenin by inhibition of GSK3 beta, GSK by stability of the beta-catenin by the inhibition of this enzyme GSK3 beta and elevated TCF4 present in the cell because of that RNA also is modified and the beta-catenin mediated transcription is facilitated. So, this is what happens that all the 3 stages receptor level, co-binding partner level, and the target gene level.

So, c-MYC is a key oncogenic target of WNT beta-catenin signaling and is also induced by the YTHDF itself because the c-MYC RNA is produced in the nucleus at a accelerated level. Not only it is produced at an accelerated level, it is translated also at an accelerated level. So, these things work together to create a cancerous environment such as gastrointestinal cancer.

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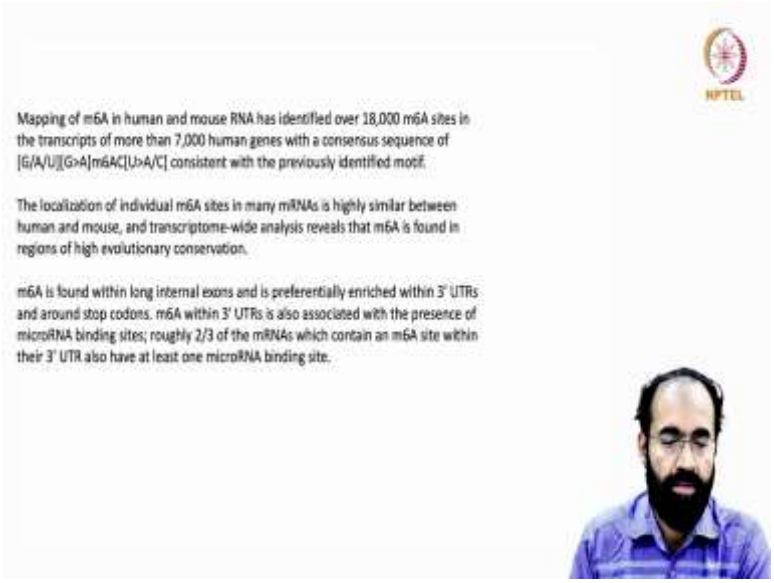

So, the mapping of m6A in human and mouse RNA has identified over 18,000 m6A sites in the transcripts of more than 7,000 human genes. It is a huge number, like more than 18,000 adenosines have been identified in 7,000 human genes with a consensus sequence of GAU as a first base like it can be either G, either A, either U, one. And the second base has to be G or A, preference is given to G over A.

And this next base comes m6A that is adenine is must which will be methylated followed by AC and the next base is U or A or C, but U is preferred more than A or C. So, this is what you find the sequence it can be GA or U here and then preferably G here then must be A followed by a C, and then it can be U technically or it can be tolerated by A or C as well, but U is preferred.

So, this is very consistent wherever such a sequence is there and which is a very plausible sequence it is not a rare sequence or something. You can always find a G, G, A, C, U sequence because it is a small stretch of sequence. So, the localisation of individual m6A sites in many mRNAs is highly similar between human and mice, suggesting an evolutionary conservation.

And the transcriptome wide analysis reveals that the m6A is found in regions of high evolutionary conservation. So, what it suggests you that such a mechanism of regulation is not randomly originated. It is evolutionarily conserved.

Many a times such a conserved mechanism indicates not that the system wants cancer. So, we should not assume that every animal have got the propensity of getting cancer because of this residues for the methylation of the adenosine residue are conserved across human and mice.

It simply says that these RNA, even if their levels are low, they should be facilitated for the translation. They should be stable enough to proceed up to translation level. But who is the culprit? The accelerated writers are the culprit to blame. If there is if there is, like if I can give a simple example if there is gold or cash kept in a counter that is for using, gold maybe for financial stability or maybe making ornaments.

If a cash is kept in a bank’s counter; that means, to give to the rightful person. That does not mean that you can go and grab it. So, I wanted money, so I just went. So, you will end up in jail.

So, presence of adenosine residues conserved adenosine residues indicates that this methylation is normal and should be promoted, should be there it is the way of living for several RNA. However, there is more and more of writing happens that is the methylation residue is there, does not mean that it should be methylated. Rather it should not be over-methylated. Over-methylated means every time, every residues methylated for a prolonged time.

So, whom to be blamed? It has to be blamed for the over-action of the writers. So, writers also important, but the over-action, you may remember that we talked about the telomerase activity. Now, somatic cells do not have telomerase activity.

So, if telomerase activity can be prolonged due to you know certain diet or maybe you can enhance you do not want an indefinitely active telomerase that can be problematic eventually. But existing telomerase if you can activate it, you can extend the length of the telomere and hence you can delay the cellular senescent.

Similarly, the writers this methyl transferase enzymes, if they are present, it is quite good. But if they are present more than required, just the opposite of telomerase what we are talking about, telomerase presence of activity is facilitating for a longer lifespan here the higher methyl transferase action can do the opposite. Many a times many signaling pathways can be up-regulated.

So, m6A is found within long internal exons and is preferentially enriched within the 3 prime and translated region and also found around the stop codons. Every RNA has got a stop codon which we already know and in and around that you are going to have the methylation. And m6A within the 3 prime UTRs is also associated with the presence of microRNA binding sites.

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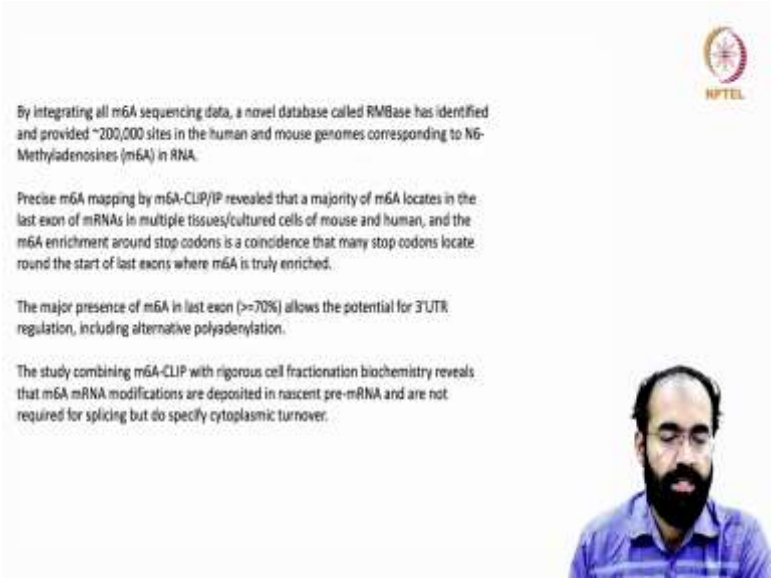

MicroRNA when it is bound, it will mark the mRNA not capable of translation and it will block the translation. Roughly, 2 out of 3 of mRNAs, if you have 3 mRNA, 2 of them of the mRNAs which contain an m6A site within their 3 prime UTRs also have at least one microRNA binding site. So, it is a strong correlation.

If there is a m6A methylation possible majority of them, two by third of the total population of microRNA also have not microRNA mRNA also have a microRNA binding site. So, by integrating all the m6A sequencing data because the detection of this m6A prevalence in mRNA population is very much possible because we have lot of sequencing methods available to detect them.

A novel database called RM base has identified and provided around 200,000 sites in the human and mouse genomes based on this prediction, like we already saw the consensus sequence and how many mRNA's have this and how many possible binding sites are in those mRNA.

So, around 200,000 binding sites are present in human and mouse genomes corresponding to the m6 methyl adenosines, that is m6A adenosines in the consensus region. Not that just random adenosine, it should match the consensus sequence as you discussed in the previous slide.

So, the precise m6A mapping by m6A CLIP IP so, it is a technique used to detect it. It has revealed that a majority of m6A locates in the last exon, last exon means near like that also contains the stop codon of mRNA's and that is seen in a variety of tissues, and no matter whether it is a native cell or a cultured cell and of both mouse and human. So, last exon have got a higher affinity to get methylated and especially it will be either in the 3 prime untranslated region or in the near the stop codon.

And the m6A enrichment around the stop codons is a coincidence that many stop codons locate around the start of last exons where the m6A is truly enriched. So, mere presence is not just there, but it also gets enriched with methylation of those adenine residues. So, the major presence of m6A in the last exon which is more than 70 percent allows the potential for the 3 prime untranslated region regulation including alternative polyadenylation.

So, alternative polyadenylation we kind of discussed that a given mRNA will have multiple polyadenylation signals can be there, and that will invite or exclude a microRNA binding site. It can also include or exclude a given secondary structure, and sometimes it can also facilitate the alternative splicing.

So, the study combining m6A-CLIP with rigorous cell fractionation biochemistry reveals that m6A mRNA modifications are deposited in nascent pre-mRNA and are not required for splicing, but to specify cytoplasmic turnover. So, we should understand that normally these modifications are a need. However, overdoing of these modifications often tweak the pathways in the favour of cell proliferation and this often can lead to lot of trouble in the form of various diseases.

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So, m6A is susceptible to dynamic regulation because we know writer causes a methylation, reader makes the RNA to get translated and make it stable, and the eraser does the opposite, it will restore back the normalcy. So, dynamic regulation happens throughout the development and homeostasis of an organism.

So, both throughout the development and in response to various cellular stimuli cellular stimuli basically means like an organism developing organism have to respond to various hormonal inflexors, nutritional inflexors or cellular demands such as you know there has to be increased cell proliferation as part of growth of an individual etcetera. So, this is called cellular stimuli.

So, analysis of m6A in mouse brain RNAs that total RNA from the mouse brain reveals that the m6A levels are low during the embryonic development and it increases dramatically by adulthood that is a time when the brain creates lots of connections. So, we have to assume the evolutionally concerned presence of m6A sites in various RNA is a necessity as a part of the organism's development and growth, but when it is not controlled effectively it can lead to complications.

So, additionally the silencing of m6A methyltransferase significantly affects gene expression and alternative mRNA splicing patterns resulting in the modulation of p53 signalling pathways and apoptosis means a cellular homeostasis require this methylation of specific adenosine residue. If you prevent it by blocking the methyltransferase, m6A

methyltransferase you end up having lots of troubles with the normal signalling pathway through p53 and which in turn can affect the cellular apoptosis.

So, m6A is also found in the on the RNA components of R-loops in human cells. You may remember the R-loops which is led to the discovery of the introns that the portions which do not pair which will loop out and they are called R-loops where it is here it is involved in the regulation of stability of RNA DNA hybrids.

Many situations there are RNA DNA hybrids even during the transcription of a gene itself RNA DNA hybrids are a necessity, and many a times RNA DNA hybrids are stable and only the RNA's H can degrade the RNA component of the RNA DNA hybrid.

So, the RNA DNA hybrid formation is a normal way of living and functioning for a given cell, but it is m6A mode modifications is seen in the RNA part of the R-loops that is projecting from this non-pairing part. That is what is forming non-pairing part in an RNA DNA molecule; there also you can see a lot of enrichment of the m6A modification.

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So, the importance of m6A methylation for various physiological process was very recently been characterized and demonstrated. The inhibition of m6A methylation via pharmacological inhibition of cellular methylations or more specifically by siRNA

mediated silencing of which enzymes m6A methylases, such as Mettl3, this has led to the elongation of the circadian period. That is another implication.

We know what is circadian period like, every time no matter like if you travel to the other side of the planet. Like, say you are travelling to USA, you will realize because now daytime, it is night for them. So, if you travel you will reach within few hours of travel, you will reach to the other side of the planet, but there when it is night you will be awake because it is your daytime and it will take at least a week's effort for you to switch back into your sleeping schedule.

So, this is a simplistic way you can say the circadian rhythm because here by evening say by around 9 o'clock or 10 o'clock, you will feel sleepy and that is how you are tuned. So, if you continue to have this rhythm say 9 or 10 o'clock you are sleepy, but when it is 9 o'clock here for you it is around 10:30 or 11 o'clock daytime at USA.

So, what will happen if you travel to USA? By around 11 o'clock of their daytime you will start feeling sleepy because that is night time for you. And by around evening of US, by around 7 o'clock or 8 o'clock in the evening you will be fully awake and you are up and running until the whole night. So, this is something to do with the circadian rhythm.

Although your environment says it is dark or your environment says it is day, your brain says other way around. So, this happens with a lot of your other features also like whether you fall asleep, no matter even if you are in a room without a clock or something, in that time when you are supposed to fall asleep you will fall asleep; without even like.

Many such experiments have done with the human volunteers, you simply get locked inside your circadian rhythm kind of prevails and light plays a major role like light when it is hitting in your retina, it can trigger the formation of you know a hormone called melatonin. So, which is important for creating a congenial situation for inducing sleep.

So, time being you understand the circadian rhythm is a periodic way where your 24 hour cycle is set in your brain. So, you will see an elongation of the circadian rhythm; that means, you are not ending the day a time when it is supposed to be ending or you are not ending a night when it is supposed to be ending, you are prolonging it.

So, in contrast, the over expression that is the down regulation. The over expression of this methylases Mettl3 led to a shorter period that is reducing the circadian rhythm. So, the mammalian circadian clock composed of transcription feedback loop tightly regulated to oscillate with a period of about 24 hours. We all know that human leave in a day of 24 hours which is the time taken for completing one day. So, it is therefore, extremely sensitive to perturbations in m6A dependent RNA processing.

So, what you understand, the down regulation of these methylases or over (Refer Time: 24:50) expression of these methylases does exactly as predicted that will screw the circadian rhythm. The down regulation of methylases prolong your circadian rhythm, over expression of methylases reduce your circadian rhythm.

So, you can guess it. If you over expression methylase naturally more RNA residues will be methylated. And what will cause? They will do over function. They will perform much stronger this they will get translated and because of this you will have a shorter time means this RNA should have been translated in a fixed amount of time.

Now, because of this with higher methylation you are having a high production in low time. That means you are eating breakfast, lunch, and dinner together, that is not possible. You can imagine what is going to happen; you will have a expanded belly. So, this is what happens, if you overexpress it more RNA will be translated, and as a result the whole process is done in shorter time. So, that is why you are shortening the circadian rhythm.

So, the mammalian circadian clock composed of transcription feedback loop tightly regulated to oscillate within a period of 24 hours and therefore, it is extremely sensitive to perturbations in the m6A dependent RNA processing. It is likely due to the presence of m6A sites within the clock genes transcripts or clock genes mRNAs.

So, the effects of global methylation inhibition what is done in this experiment on the circadian period, in mouse cells can be prevented by ectopic expression of an enzyme from the bacterial methyl metabolism. That is one way of rescuing it. So, if you knock down the methylases you are having a prolonged circadian rhythm, but you can counter it by using an enzyme that is part of the bacterial methyl metabolism.

So, mouse cells expressing bacterial protein were resistant to pharmacological inhibition of the methyl metabolism that shows no decrease in the mRNA m6A methylation or protein methylation.

So, what we can understand that some of the components of the bacterial metabolism can act as a rescuer for this methylation perturbations, also suggest that these mechanisms are highly plastic, and they work across the species, and these mechanisms are a way of living for various biological systems. I will continue about these RNA modifications and also protein translations in the next class.

Thank you.