International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Nonsense Mediated mRNA Decay (NMD) Mechanism in *Drosophila*: A Short Review

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Abstract: ***Nonsense-mediated mRNA decay (NMD) is a mechanism that prevents the accumulation of malfunctioning protein and also regulates the clinical manifestation of many genetic disorders. This pathway not only degrade mRNA containing frame shift and nonsense mutation but also regulate the expression of naturally occurring transcript having features recognized by the NMD machinery.NMD contributes to the post transcriptional regulation of about 10% of the transcriptome in Drosophila, yeast and human cell. In Drosophila, the degradation of non-sense transcript is initiated by endonucleolytic cleavage near the PTC and RNA fragments are degraded from the newly generated ends. The 5'fragmentis degraded by exosome and 3' fragment is degraded by Xrn1. In Drosophila, many proteins involved in the mechanism of NMD mainly are SMG1, SMG5, SMG6, UPF1, UPF2, UPF3. For the activation of NMD, recognition of pre-mature termination codon PTC is necessary. In this review we have discussed the current knowledge of NMD mechanism in Drosophila that will help us in future studies.***

Keywords: NMD, Nucleus, UPF1, *Drosophila*, Ribosome

1. Introduction

Nonsense mediate mRNA decay (NMD) is a mechanism that detects and degrades the mRNA which contains premature translation termination codon (PTC).This PTC makes abnormal and harmful protein. At this point NMD safeguards cells, accumulate and delete the abnormal protein to improve the expression of gene [1, 2]. It is a mechanism that also differentiates PTC and natural stop codons [3].This mechanism is involved in the rapid degradation of mRNA whose translation is not terminated properly by a ribosome. In this way, NMD contributes to the degradation of abnormal mRNA to improve the fidelity of gene expression and also to regulate the gene expression at the post transcriptional level [4]. PTC occurs in mRNA at both DNA and RNA level. At DNA level it occurs due to mutation in genes and at RNA level due to alternative splicing or transcriptional error [5]. .NMD regulates the expression of gene in yeast, fruit fly (*Drosophila*) and human and also plays a role in biological processes such as cell cycle, cell proliferation, cellular transport, telomere maintenance and metabolism [6]. Initially, NMD recognizes the PTC and then triggers the degradation. If the ribosomes are unable to terminate translation properly or the open reading frame gets truncated, an error occurs in gene expression that leads to introduction of PTC. NMD can also be initiated by deadenylation, decaping or endonucleolytic cleavage near the PTC [4]. In *Drosophila*, PABC1 is a basic determinant for PTC [3]. Recognition of PTC in yeast and *Drosophila* is independent of an intron and RNA splicing [7]. Downstream sequence elements within the coding region are required to trigger NMD in yeast and *Drosophila* [8]. NMD mechanism requires some factors including UPF1,UPF2,UPF3, SMG1, SMG5, SMG6 and SMG7 called SMGs [9,10,11].UPF proteins develop the core of NMD machinery whereas UPF1 is an important factor for NMD [12,13]. The SMG1 protein catalyzes the phosphorylation of UPF1 at serine residues [14].SMG5, SMG6 and SMG7 are involved in the dephosphorylation of UPF1 [12] and SMG7 is absent in *Drosophila* [15].

2. Activation of NMD Mechanism

In *Drosophila*, activation of NMD mechanism depends on PTC recognition. Factors that recognize the PTC and activate the NMD mechanism includes:

- Proper interaction of 3UTR associated proteins with ribosome.
- Length of 3UTR.
- Binding of PABC1.

2.1 Proper interaction of 3'UTR associated proteins with ribosome

For efficient termination, ribosome must interact with 3'UTR associated protein. In case of natural stop codon, the ribosome interacts with 3'UTR associated proteins and terminates the translation efficiently without any need of degradation. However, if PTC is present, the ribosome cannot interact with the 3'UTR associated proteins as a result the termination is impaired or slow and is responsible for the activation of NMD mechanism that start the process of degradation [16].

2.2 Length of 3'UTR

The activation of NMD also depends on the length of 3'UTR.The length of 3'UTR is also responsible for activation of NMD and initiation of degradation process [3]. Where transcripts have an exceptionally natural long 3'UTR the NMD does not get activated [2].

2.3 Binding of PABC1

Binding of poly A binding protein cytoplasmic 1 (PABC1) to the downstream sequence of promoter region is necessary for the NMD activation because its depletion inactivates the

NMD and translation efficiency is not affected [17].The binding of PABPC1 and cleavage and polyadenylation reaction also provide positional information for PTC recognition [19].To explain this point scientists designed an ADH reporter (alcohol dehydrogenate) whose polyadenylation and cleavage signals are replaced by histone H3 stem loop structure [18] or by the ribozyme elements [20]. Later on, they observed that the reporter bearing a PTC at codon 64 is not regulated by NMD. For the regulation of PTC at 64th codon, scientist artificially inserted adenosine residue upstream of the histone loop or ribozyme elements and after getting the poly A signal, NMD mechanism was activated. It was thus concluded that binding of PABC1 is necessary for NMD activation.

3. Working of NMD Pathway

As discussed above, many proteins are involved in the NMD pathway. The process of NMD occurs in nucleus and advances in the cytoplasm. At first, UPf3 interacts with the components of exon junction complex (EJC) and loaded on mRNA during the process of splicing (removal of introns). After the binding of UPf3 and its export into the cytoplasm, UPf2 joins this complex [21]. During the process of translation, the complex of UPf2, UPf3 and EJC is displaced by ribosome as they cross the mRNA. If a translating ribosome encounters a stop codon upstream of an EJC, it results in binding of SURF complexes comprising SMG1, UPF1 and the peptide releasing factors e RF1 and e RF3 to the ribosome [22]. SMG1 phosphorylates the UPF1 and leads to dissociation of eRF1 and eRF3 which triggers the mRNA decay. Following that the dephosphorylation of UPF1 is mediated by Smg5 and SMG6. Although, Smg5-7 are not phosphatases themselves, they are thought to trigger UPF1 dephosphorylation by recruiting protein phosphatase 2A (PP2A). This model is based on the observations that Smg5 and Smg7 interact with each other and are part of a larger complex comprising phosphatase PP2A and dephosphorylate the Upf1[23,24].Similarly, Smg6 is part of a protein complex comprising PP2A and dephosphorylate the Upf1[25]. With the exception of Smg7, *Drosophila* orthologs to core components of the NMD machinery have been identified, including Upf1, Upf2, Upf3, Smg1, Smg5 and Smg6. Depletion of these proteins by RNAi in *Drosophila* Schneider cells stabilizes various PTC-containing mRNAs, providing evidence for a role in NMD[15].

4. Conclusion and Future Perspectives

In this brief review, we have discussed the role of NMD in *D.melanogester*, several conserved effectors and how these factors play a key role in non-sense mediated decay mechanism.

We focused on mechanistic aspect of target mRNA identification and degradation in *Drosophila*. In fruit fly (*Drosophila*) NMD target is initiated by endonucleolytic process and it does not require exon junction elements. We summarized the role of exon- exon junction complexes and function of UPF1, SMG5, SMG6 and SMG7 in promoting mRNA decay through different routes.

However, we still lag behind in understanding the NMD effectors interaction and it's leading into this process. We also lack in details of endonucleolytic process in *Drosophila* and functional relationship between UPF1, SMG5, SMG6 and SMG7.These topics can be subject of active research in the future.

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