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Hypothesis

Nucleosome deposition and DNA methylation may participate in the recognition of premature termination codon in nonsense-mediated mRNA decay

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ABSTRACT

In non-mammalian eukaryotes, an abnormally long 3′ untranslated region (UTR) is generally thought to be the definitive signal in the recognition of a premature termination codon (PTC) in nonsense-mediated mRNA decay (NMD). However, because the lengths of 3′ UTRs in normal mRNAs are widely distributed, "abnormally long" is hard to define. Distinct peaks of nucleosome deposition and DNA methylation have recently been found at coding region boundaries. We propose that nucleosomes and DNA methylation just upstream of a normal stop codon are ideal indicators for the position of a normal stop codon and may thus serve as signals in PTC recognition.

1. Premature termination codon (PTC) recognition: a long 3′ untranslated region (UTR) is not enough

In a living cell, replication, DNA repair, transcription and splicing are never 100% accurate. A certain fraction of erroneous mRNA transcripts are inevitably produced. Eukaryotes have evolved multiple quality control systems to recognize and eliminate these transcripts before they produce faulty proteins [1]. One of the most extensively studied systems is nonsense-mediated mRNA decay (NMD) [1–4], which recognizes and degrades transcripts that harbor PTCs. These aberrant mRNAs, if not degraded, will produce truncated peptides, many of which often have dominant-negative functions through competition with their full-length normal protein counterparts [5].

The first step in NMD is distinguishing PTCs from normal stop codons. Because the PTCs are also composed of normal nucleotides, additional signals are required to identify them as premature.

In mammals, multiple proteins, known as the "exon–exon junction complex" (EJC), are deposited on the exon–exon boundaries of mRNA transcripts during splicing. The EJCs provide natural markers for different positions in an mRNA transcript. Normal stop codons are primarily located in the last exons [1]. Thus, the last EJC, which would be just upstream of most normal stop codons, is an ideal signal for "close to the end of the coding sequence" in mRNA transcripts. Nonsense codons that are located >55 nucleotides upstream of the last exon–exon junction have been found to elicit NMD in mammalian cells [1].

However, in the yeast *Saccharomyces cerevisiae*, most genes lack introns. Thus, distinguishing PTCs from normal stop codons in yeast would not involve EJCs. Similarly, PTC definition occurs independently of exon–exon junctions in both *Caenorhabditis elegans* and *Drosophila melanogaster* [6,7]. In these organisms, a PTC is generally believed to be defined by its distance from the 3′ end of the mRNA (i.e., the length of the 3′ UTR) [1,2,8].

The idea that abnormally long 3′ UTRs act as the determining factor of PTC recognition has a flaw, especially for those organisms that do not use EJCs as an essential enhancer. The lengths of 3′ UTRs in normal mRNA transcripts are widely distributed in *S. cerevisiae*, *C. elegans* and *D. melanogaster* (Fig. 1). In a yeast gene with a short 3′ UTR (e.g., 100 nucleotides), a PTC that is 80 nucleotides upstream of the normal stop codon results in an abnormal 3′ UTR with a length of just 180 nucleotides. Because this abnormal 3′ UTR is shorter than approximately 50% of all normal 3′ UTRs in yeast, it could hardly be considered to be "abnormally long". Even considering the observation that the expression of some genes is down-regulated by their long 3′ UTR through NMD [8], it is still difficult to imagine that a PTC is specified simply by the length of an abnormal 3′ UTR.

One possible solution of this problem is that the normal 3′ UTRs with different lengths, by altering the spatial configuration, may produce similar physical distances from the stop codons to the poly(A) tails or their binding proteins. In recent years, accumulat-

Abbreviations: NMD, nonsense-mediated mRNA decay; PTC, premature termination codon; UTR, untranslated region; EJC, exon–exon junction complex; DSE, downstream sequence element

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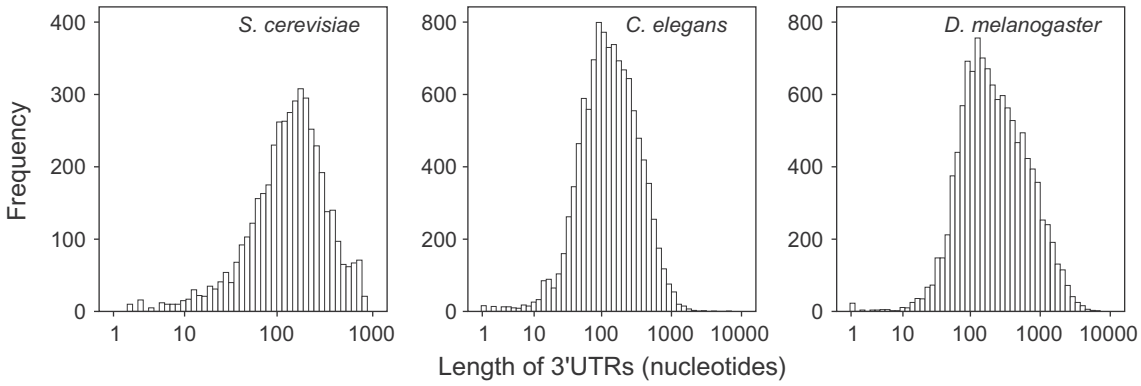

Fig. 1. Length distribution of 3′ UTRs in three non-mammalian eukaryotes. The data from *S. cerevisiae* were obtained from a previous publication [40], those from *C. elegans* from WormBase (release WS211) and those from *D. melanogaster* from FlyBase (FlyBase r5.26).

ing evidence indicates that the physical distance between stop codon and poly(A)-binding proteins (Pab1p and its ortholog PABPC1) is a conserved determinant for PTC recognition in eukaryotic cells [9–14]. However, there are also some yeast mRNAs whose NMD processes do not require a poly(A) tail or its binding proteins [15,16]. So, the story is not complete. There should exist additional signals for PTC recognition. Here, we propose that nucleosome deposition and DNA methylation just upstream of the normal stop codon may serve as additional signals in PTC recognition.

2. Nucleosome deposition is versatile

In the nucleus of a eukaryotic cell, the DNA wraps around a central core of eight histone proteins to form a subunit of chromatin called a nucleosome. Nucleosomes are not just inert building blocks of chromatin; for example, chemical modifications of histones can regulate gene expression by changing the accessibility of genes [17]. By analyzing nucleosome distribution across genes, researchers have recently found that nucleosomes are strongly enriched on exons, especially those flanked by long introns [18–24]. Thus, nucleosomes could serve as potential markers to distinguish small exons from large introns during splicing. More strikingly, nucleosome positioning has been reported to identify the position of DNA replication origins in *S. cerevisiae* [25]. Previously identified motifs have been shown to be insufficient to account for the proper recognition of both splice sites and DNA replication origins, and nucleosome positioning fills this gap.

The versatility of nucleosome deposition suggests its possible role in other processes in which the required signals have not yet been fully revealed, such as PTC recognition in NMD of non-mammalian eukaryotes, the recognition of different genes after transcription of an operon in *C. elegans* and the recognition of trans-splicing partners.

3. Nucleosome deposition and DNA methylation: ideal indicators for the position of a normal stop codon

Could nucleosome deposition function in PTC recognition? A PTC is an abnormal stop codon upstream of the normal stop codon. Theoretically, the most robust way to distinguish a PTC from the normal stop codon is to directly identify the exact position of the normal stop codon and then determine whether the target stop codon is upstream of that position. If there is no inherently direct indicator for determining the exact position of a normal stop codon, other indicators that closely flank normal stop codons may serve as appropriate substitutes. In mammals, the last EJC on an mRNA transcript meets such a requirement and acts as the essential signal for PTC recognition. In non-mammalian eukaryotes, for the nucleosome to act as a signal for PTC recognition it must be preferentially deposited near the normal stop codon. Indeed, this pattern has recently been reported.

Choi and co-workers [26] found that there are distinct peaks of nucleosome deposition and DNA methylation at both ends of protein-coding sequences: one just downstream of the start codon and the other just upstream of the stop codon. They also observed that the peak near the stop codon is generally higher than that near the start codon. Given that the nucleosomal peaks were observed in humans, flies and yeasts and the methylation peaks were observed in humans, mice and rice [26,27], the nucleosomal/DNA methylation peaks near the coding boundaries appear to be conserved in all of the major eukaryotic branches. Most recently, Chodavarapu et al. found that nucleosomal DNA was more highly methylated than flanking DNA, which indicates that DNA methylation pattern is determined by nucleosome positioning [28]. The nucleosomal peaks and DNA methylation peaks near the coding boundaries might be two sides of the same coin.

The inherent location of both the nucleosomes and the DNA methylation just upstream of normal stop codons fulfills the requirements needed to act as signals in PTC recognition. Nucleosome deposition and/or DNA methylation just upstream of the normal stop codon could likely elicit the recruitment of some special protein components of mRNP during transcription. After being exported to cytoplasm with mRNA, these proteins would then indicate the position of the normal stop codon during translation. However, if the pioneer round of translation finishes before reaching these proteins, the mRNA decay process would be activated. In this hypothesis, nucleosome deposition and DNA methylation just upstream of the normal stop codon serve as the indicators for the position of the normal stop codon, and stop codons located away from these epigenetic signals are recognized as PTCs.

4. Testing the hypothesis

Whether DNA sequence preference is the main determining factor of nucleosome deposition is still under debate [29,30]. Even if DNA sequence preference contributes little to global nucleosome deposition, it may still be the determining factor for nucleosome deposition at specific sites, such as the coding region boundaries. If our hypothesis is true, replacement of the nucleosome/DNA-methylation-determining region by other sequences while maintaining the position of the normal stop codon would cause the normal mRNA transcript to become susceptible to NMD (Fig. 2C). Furthermore, duplication of the nucleosome/DNA-methylation-determining region, normally located at the coding end, in the region upstream of the PTC, would make the aberrant mRNA transcripts immune to NMD, even though the length of the 3′ UTR

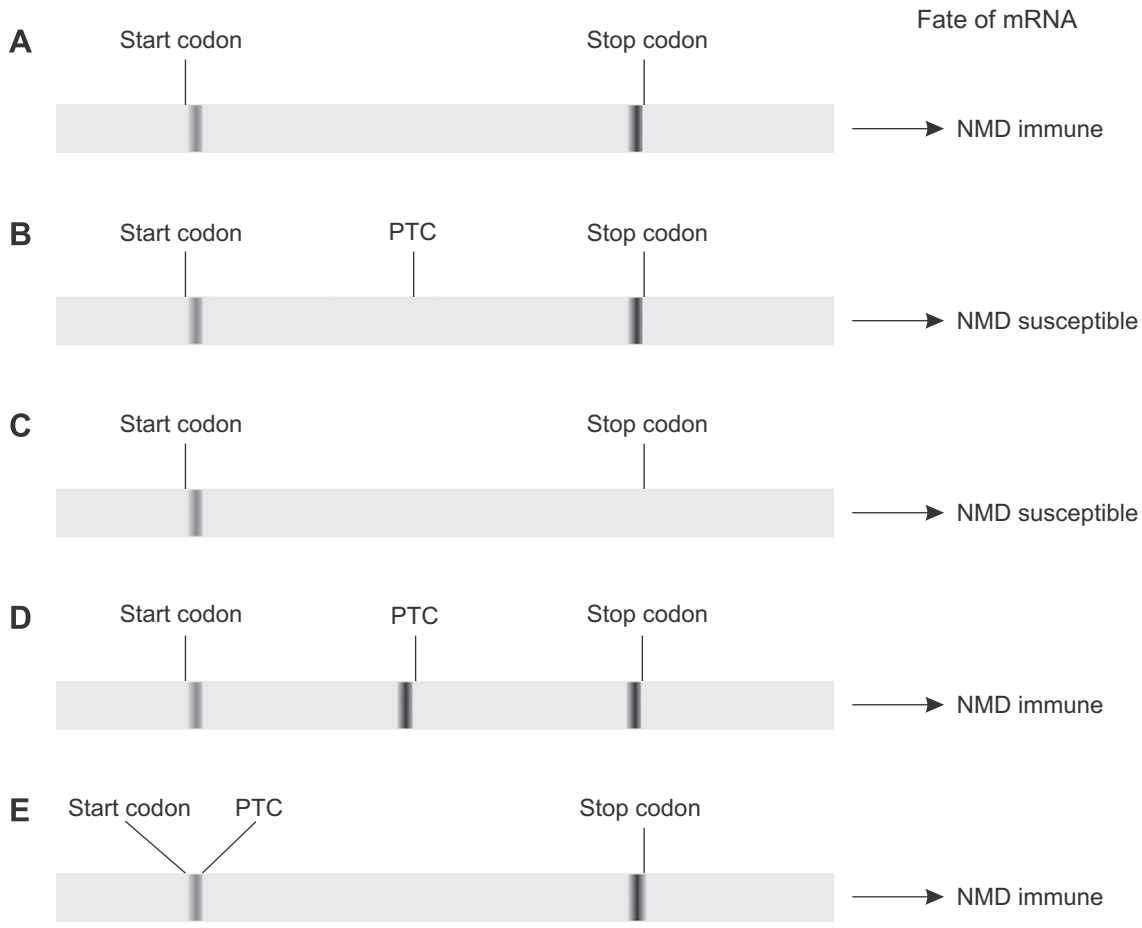

Fig. 2. Strategies to test the hypothesis presented in this paper. According to our hypothesis, nucleosome deposition and DNA methylation are markers of normal stop codon. The fates of mRNA molecules are determined by the relative positions between stop codons and nucleosome deposition and DNA methylation. Our hypothesis could be tested by analyzing whether its predictions are true. The darkness represents the density of nucleosome deposition and DNA methylation. (A) A normal gene with nucleosome deposition and DNA methylation just downstream of the start codon and just upstream of the stop codon. Its mRNA molecules are immune to NMD process. (B) An aberrant gene with a premature stop codon (PTC). Its mRNA molecules are natural targets of NMD. Our hypothesis predicts that the gene lacks nucleosome deposition and DNA methylation near the PTC. (C) An aberrant gene with its nucleosome/DNA-methylation-determining sequence near the normal stop codon removed. Its normal stop codon is recognized as a PTC during translation, and trigger NMD. (D) An aberrant gene with a PTC, but also nucleosome/DNA-methylation-determining sequence duplicated near the PTC. The PTC is marked as a normal stop codon and thus does not trigger NMD. (E) If the nucleosome deposition and DNA methylation just downstream of the start codon and those just upstream of the normal stop codon elicit similar proteins to mRNA molecules, a PTC in close proximity to the start codon would be mis-recognized as normal stop codon and thus it does not trigger NMD.

would increase (Fig. 2D). In addition, we could use the mRNA segments corresponding to the nucleosome/DNA-methylation-determining DNA regions as potential ligands to isolate the proteins that indicate the position of normal stop codon by RNA affinity chromatography [31]. Once the proteins are determined, we could test the hypothesis by knocking out their genes and checking how NMD process is affected.

Another way to test this hypothesis would be to analyze the nucleosome/DNA methylation pattern of genes with multiple alternatively spliced transcripts. Intron retention is a common form of alternative splicing. Some of the alternatively spliced transcripts resulting from splicing errors are targets of NMD [32,33], while others are immune to NMD and are able to produce functional proteins. For example, the *rem1* gene in fission yeast has two transcripts, one that is fully spliced and the other that retains an intron and contains a PTC. Although truncated, the protein produced by the intron retention transcript is functional [34]. If our hypothesis is correct, nucleosomes and DNA methylation signals should be observed just upstream of any functional PTCs in a global analysis of the nucleosome deposition and DNA methylation patterns of alternatively spliced transcripts.

Previous observations that could not be explained by older models of NMD have also suggested a means to test our hypothesis. In the traditional model of NMD, the relative position of the poly(A) tail and its binding proteins to a PTC was the critical feature for the definition of a PTC. In contrast, NMD in some yeast genes does not require a poly(A) tail or its binding proteins [15,16]. According to our hypothesis, there may be special epigenetic markers, such as nucleosome deposition and/or DNA methylation, upstream of the normal stop codon that help to define the PTC.

5. Discussion

In 2004, Amrani et al. found that in yeast, ribosomes that encountered PTCs dissociated from mRNA much less efficiently than those that stalled at normal stop codons [9]. They proposed the presence of a termination-stimulating signal near the normal stop codon [9], but the nature of this signal has yet to be discovered. Nucleosome deposition and DNA methylation at the 3′ end of the coding region might have initially evolved as epigenetic signals for the efficient termination of the translation process and were later recruited as signals for PTC recognition during the time that NMD originated. The premise behind this idea is that histones and DNA methylation predate the appearance of NMD in evolution. DNA methylation occurs in many bacterial, archaeal and eukaryotic

species [35], and histones have been identified in many archaeal and all eukaryotic species [36]. It is reasonable to assume that histones and methylation predate the origin of eukaryotes and NMD.

Because both downstream sequence elements (DSE) and nucleosomes/DNA methylation reside within the normal translational reading frame [1,37], whether they are two aspects of a single issue or whether DSEs are just special motifs that guide nucleosome deposition and DNA methylation just upstream of normal stop codons should also be tested.

As epigenetic signals, the patterns of nucleosome deposition and DNA methylation may vary with tissue type to some degree. Thus, the recognition of PTCs would have tissue specificity, a phenomenon that has already been observed [38]. As a result, some alternatively spliced transcripts would either be degraded or preserved according to tissue type.

It has been revealed that PTCs, if in close proximity to the start codon, could not efficiently trigger NMD [39]. In the frame work of our hypothesis, the nucleosome deposition and DNA methylation just downstream of the start stop codon might elicit similar proteins to mRNA molecules as those just upstream of the stop codon. Consequently, the PTCs in close proximity to the start codon would be mis-recognized as normal stop codons during translation (Fig. 2E). Although this explanation is reasonable, we are inclined to accept an alternate explanation that has some experimental supports: These PTCs has a very short physical distance to PABPC1 [13].

In short human genes (CDS < 1 kb), the nucleosomal/DNA methylation peaks have been observed not at the 3′ ends of coding sequences, but in the middle of genes [26]. Thus, the EJC is likely more important than nucleosome deposition and DNA methylation for mammalian PTC recognition. Nucleosome deposition and DNA methylation may be essential for PTC recognition in non-mammalian eukaryotes. Even in non-mammalian eukaryotes, however, we are inclined toward a conservative hypothesis – that the nucleosome deposition and DNA methylation just upstream of normal stop codons participate in PTC recognition rather than act as an exclusive signal.

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