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Nonsense shielding: protecting RNA from decay leads to cancer

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Despite intense scrutiny, the signals that determine whether a given RNA is degraded by the highly conserved and selective nonsense-mediated RNA decay (NMD) pathway remain murky. In this issue of *The EMBO Journal*, Kishor *et al* shed light on this issue by demonstrating that the RNA-binding protein, hnRNP L, protects a subset of RNAs from degradation by NMD. This mechanism is responsible for stabilizing the mRNA encoding the pro-survival "oncogenic" protein, BCL-2, in B-cell lymphoma.

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See also: **A Kishor *et al*** (February 2019)

The expression of a gene depends just as much on the stability of the mRNA it encodes as the rate at which it is transcribed. Indeed, regulation of RNA stability confers qualities not offered by transcriptional control, such as the ability to rapidly eliminate an mRNA when its gene product is no longer needed. While much has been learnt about RNA decay mechanisms, we are still largely in the dark as to the specific signals that ultimately determine their activity. There is no better example of this than nonsense-mediated decay (NMD), a highly selective RNA turnover pathway triggered by stop codons in specific contexts. It has been particularly perplexing why a given context—such as a long 3′ untranslated region (3′UTR) downstream of the stop codon—triggers NMD in some RNAs but not others. In this issue of *The EMBO Journal*, Kishor *et al* significantly fill this gap by defining a key molecule that determines whether or not a long 3′UTR elicits RNA decay (Kishor *et al*, 2019).

NMD was originally discovered through its role as a quality control mechanism that degrades aberrant mRNAs harboring premature termination codons generated by mutations, errors in splicing, and programmed gene rearrangements (Fig 1A; Nickless *et al*, 2017). Subsequently, NMD was found to also degrade subsets of normal mRNAs with stop codons in specific contexts. Increasing evidence suggests that the ability of NMD to degrade specific normal mRNAs is regulated and critical for a variety of functions ranging from differentiation and development to stress responses and autophagy. For example, NMD degrades the mRNA encoding the pro-apoptotic protein, GADD45, to allow for normal early fly development; NMD degrades *Smad7* mRNA to promote self-renewal of neural stem cells; and NMD degrades *IRE1α* mRNA to shape the unfolded protein stress response (Karam *et al*, 2013; Nickless *et al*, 2017).

To comprehend how NMD functions in its various biological roles, it is critical to be able to define all its target mRNAs. While several different "NMD-inducing signals" have been defined, only one of these *consistently* elicits mRNA decay. This signal—an intron in the 3′UTR—triggers NMD by recruiting a large set of NMD-promoting proteins, called the exon-junction complex (EJC), just upstream of splice junctions after RNA splicing (Boehm & Gehring, 2016). Because ribosomes displace all EJCs in the main open reading frame (ORF), only introns downstream of the main ORF (i.e., in the 3′UTR) elicit NMD.

Kishor *et al* focus their studies on a different NMD-inducing signal: a long 3′UTR. Originally defined as an NMD-promoting signal in yeast, long 3′UTRs were subsequently shown to also drive the rapid decay of many mammalian transcripts (Eberle *et al*, 2008; Silva *et al*, 2008). However, the field has not able to define a specific 3′UTR length that triggers NMD, making target prediction challenging. Some short 3′UTRs (< 1 kb) are capable of triggering NMD, and many long 3′UTRs (> 1 kb) do not elicit NMD (Karam *et al*, 2013; Toma *et al*, 2015).

What is the molecular basis for this heterogeneous response? Kishor *et al* demonstrate that the RNA-binding protein, hnRNP L, is critical for determining whether mRNAs harboring a long 3′UTR is degraded by NMD (Fig 1A; Kishor *et al*, 2019). The authors were initially led to this possibility when they discovered that mRNAs with long 3′UTRs are significantly enriched for hnRNP L-binding sites (CA repeats) and high hnRNP L occupancy in their 3′UTRs. To assess whether this might be relevant to NMD, they next examined the occupancy of UPF1, an RNA helicase essential for NMD. UPF1 only transiently interacts with most mRNAs, but remains bound to NMD target mRNAs, and thus, UPF1 occupancy provides a measure of the likelihood that an mRNA will be degraded by NMD. The authors found that mRNAs with high hnRNP L occupancy tended to have low UPF1 occupancy. This raised the possibility that hnRNP L *inhibits* NMD, which the authors directly showed using RNA decay assays, the gold standard for identifying NMD substrates. Kishor *et al* then mapped hnRNP L occupancy and found that the greatest protection from NMD was conferred on mRNAs with high hnRNP L density within 100 nucleotides from the termination codon. Indeed, some mRNAs with high hnRNP L occupancy in this termination region enjoyed complete NMD immunity.

Not only did Kishor *et al* identify an intriguing molecular mechanism that controls mRNA turnover, but they identified a clinical scenario—B-cell lymphoma—in

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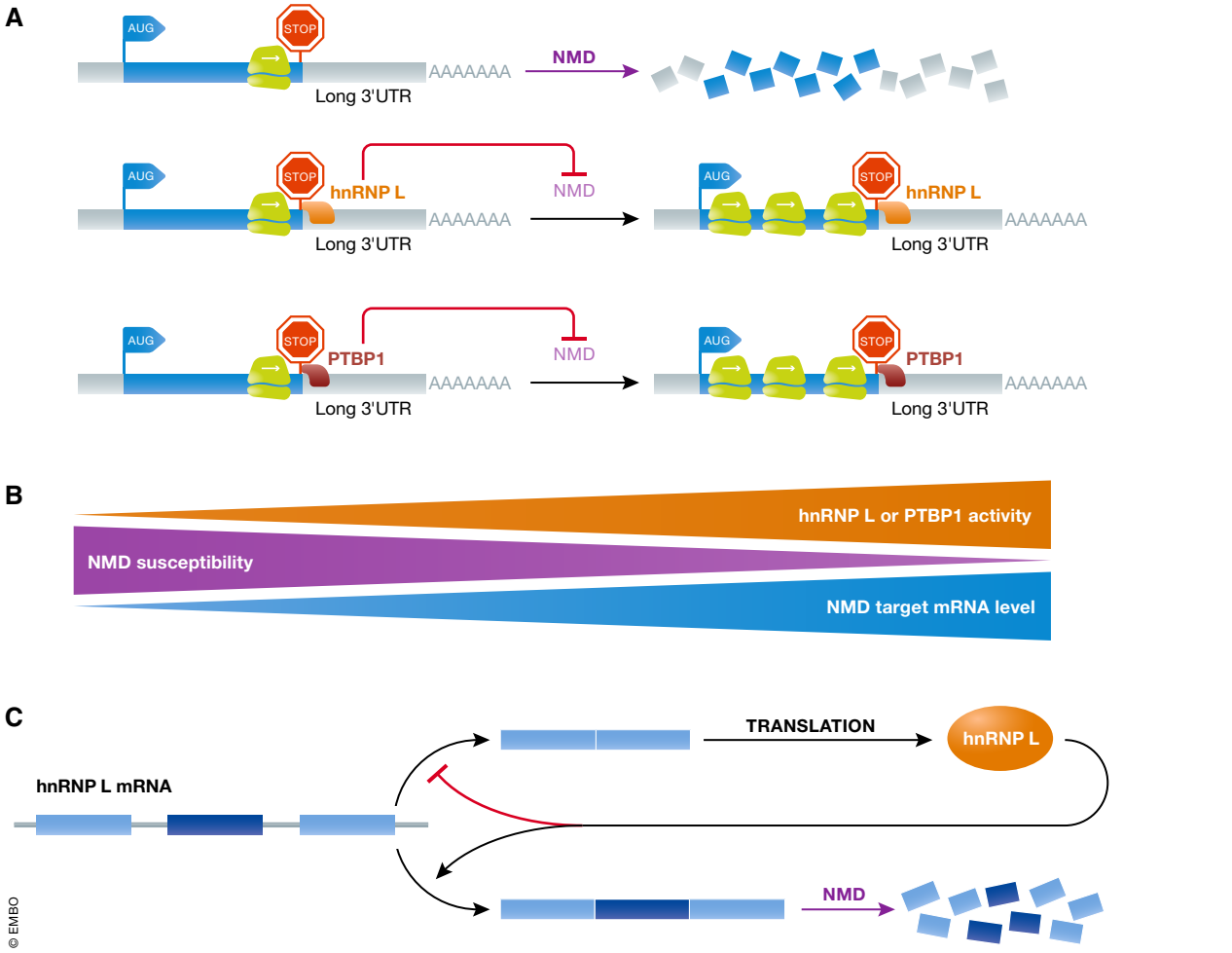

Figure 1. hnRNP L and PTBP1 protect mRNAs with long 3′UTRs from decay by the nonsense-mediated decay (NMD) pathway.
(A) mRNAs harboring long 3′UTRs are inconsistent targets for NMD. Kishor *et al* demonstrate that the 3′UTR of a subset of these mRNAs is bound and protected from NMD by hnRNP L. The same group has previously shown a similar protective mechanism for PTBP1. (B) Increasing hnRNP L and/or PTBP1 levels leads to decreased NMD susceptibility and a consequent stabilization of mRNAs harboring long 3′UTRs and binding sites for these RNA-binding proteins. (C) hnRNP L (as well as PTBP1, not shown) auto-regulates its own expression by regulating the inclusion or exclusion of alternative exons and, consequently, its own susceptibility to NMD.

which it acts. B-cell lymphomas are caused by over-expression of the *BCL2* pro-survival gene as a result of its translocation to a region near the immunoglobulin heavy chain (*IGH*) enhancer. The authors observed that the *BCL2:IGH* mRNA produced as a result of the most common class of reciprocal translocation in follicular B-cell lymphoma had multiple introns downstream of the *BCL2* ORF stop codon, which predicted that this hybrid RNA would be *destabilized* by NMD. This was surprising, as this would reduce the selective advantage conferred by BCL2 to such tumors. It turns out, however, that NMD's protective role was overridden by numerous hnRNP L-binding sites near the *BCL2* stop codon. Several lines of evidence supported this, including loss of NMD protection when the hnRNP L-binding sites were removed from *BCL2:IGH* using CRISPR/Cas9. Consistent with reduced BCL2 expression, this deletion also increased lymphoma cell apoptosis *in vitro*. In the future, it will be important to determine whether hnRNP L promotes the tumorigenicity of lymphoma cells *in vivo*. If so, hnRNP L will become a prime therapeutic target for treating lymphoma and other diseases caused by genes that are immune to NMD as a result of hnRNP L-binding.

The same group previously published that another hnRNP protein—PTBP1 (originally called "PTB")—also acts to protect mRNAs harboring long 3′UTRs from NMD (Fig 1A; Ge *et al*, 2016). Following up on this, Kishor *et al* showed that when they examined *both* hnRNP L- and PTBP1-binding sites as criteria, they could successfully predict NMD target mRNAs with high specificity. The hnRNP family is large, and thus, it will be interesting to see whether other family members also suppress NMD. Indeed, Kishor *et al* provide hints that hnRNP C and hnRNP U may confer NMD immunity.

Why do hnRNP L and PTBP1 confer NMD immunity? One possibility is this property is a remnant of an evolutionary strategy to allow high expression of genes with long 3′UTRs. Long 3′UTRs provide a plethora of opportunities for regulation (e.g., via microRNAs and specific RNA-binding proteins), and thus, it is likely there was strong selection for this

property during evolutionary time. For mRNAs with long 3′UTRs that required high expression, hnRNP L- and PTBP1-binding sites may have co-evolved to avoid activating NMD.

A non-mutually exclusive explanation for why hnRNP L and PTBP1 confer NMD immunity is to dynamically regulate the expression of batteries of mRNAs in specific physiological contexts (Fig 1B). Despite its ubiquitous expression, hnRNP L is known to be subject to regulation (e.g., by phosphorylation), which would be predicted to shift the degree of protection it affords to NMD target RNAs with hnRNP L-binding sites. The consequent changes in the levels of these mRNAs could then have biological consequences for hnRNP L-regulated functions, including in hematopoiesis, angiogenesis, and hypoxic responses. In the case of PTBP1, it is downregulated in response to neuronal differentiation signals, which is critical for efficient neuronal differentiation. It will be intriguing to determine whether PTBP1 downregulation acts to blunt NMD immunity conferred on one or more key NMD target mRNAs in order to drive neural differentiation.

A final twist in this story is the previous finding that hnRNP L and PTBP1 are both subject to autoregulation by NMD (Fig 1C). High levels of these proteins lead to a shift in the splicing of their respective mRNAs that makes them susceptible to decay by NMD, thereby forming a negative-feedback circuit (Rossbach *et al*, 2009; Wachter *et al*, 2012). Thus, not only do these RNA-binding proteins regulate NMD, but they themselves are regulated by NMD. In the future, it will be important to determine whether this is a novel type of circuitry specific to a few specialized circumstances or whether, instead, NMD is deeply entrenched in a wide variety of biochemical pathways.

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