PERSPECTIVE

Nonsense-mediated RNA decay: at the 'cutting edge' of regulated snoRNA production

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Production of multiple functional RNAs from a single primary transcript is an extremely efficient use of genetic information, although it complicates the ability of the cell to independently regulate the production of each RNA. For the case of small nucleolar RNAs (snoRNAs) encoded within introns of mRNA genes, Lykke-Andersen and colleagues (pp. 2498–2517) demonstrated that alternative splicing and the SMG6 endonuclease of the nonsense-mediated RNA decay pathway are key regulators that control which RNAs accumulate.

Protein-coding and noncoding RNAs (ncRNAs) are expressed through a complex, coordinated network in human cells. Most genes are able to generate multiple mRNA isoforms due to alternative splicing and polyadenylation events, but the story does not end there. Some genes encode both mRNAs and regulatory small RNAs from the same primary transcript, raising the question of how differential production of coding RNAs and ncRNAs from this single precursor can be achieved. The study by Lykke-Andersen et al. (2014) in this issue of *Genes & Development* illustrates how the nonsense-mediated decay (NMD) quality control pathway is one way the cell solves this problem by selectively degrading certain alternatively processed RNAs.

Most RNA degradation in cells is initiated by modifying or "marking" the transcript so that it becomes accessible to exoribonucleases. This modification can be removal of the 5′ cap (decapping), endonucleolytic cleavage in the body of the mRNA, or shortening of the poly(A) tail (deadenylation), all of which act to prevent translation. NMD is best characterized as the pathway removing mRNAs that contain a premature termination codon (PTC) (Popp and Maquat 2013). When a ribosome encounters a termination codon, particularly on mRNAs with long 3′ untranslated regions or transcripts still bound to the exon junction complex, the helicase UPF1 is recruited. UPF1 is then phosphorylated and recruits several factors, including the SMG6 endonuclease and SMG5/SMG7 that recruit additional RNA decay factors, including deadenylases and decapping enzymes. NMD thus causes the mRNA to experience one of three events—endonucleolytic cleavage, 3′ deadenylation, or 5′ decapping—targeting it for exonucleolytic decay. How and why the NMD machinery decides which "mark" to place on the mRNA, however, is unknown.

To reveal new insights into how NMD shapes the poly(A)$^+$ (or nondeadenylated) portion of the transcriptome, Lykke-Andersen et al. (2014) designed an innovative sequencing strategy to quantify RNA 5′ end decay intermediates. Depletion of XRN1, the main 5′–3′ cytoplasmic exonuclease, stabilizes mRNA decay intermediates, thus increasing their abundance and facilitating detection and quantification by deep sequencing. In addition, the mechanism by which each decay intermediate was generated can be inferred by incorporating additional data sets. CAGE (cap analysis of gene expression) tags, which mark the 5′ ends of capped RNAs, allowed putative decapping events to be identified, while codepletion of XRN1 with either SMG6 or UPF1 revealed NMD-specific events.

Although NMD triggers decapping of some mRNAs, deep sequencing revealed that endonucleolytic cleavage by SMG6 plays the predominant role in triggering degradation of human nonsense-containing mRNAs that do not undergo deadenylation. These endocleavage sites cluster close to the PTC, suggesting that the NMD-eliciting complex probes the substrate for nearby accessible regions. Interestingly, when SMG6 was depleted, decapped mRNAs accumulated. This suggests that although cleavage

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by SMG6 is kinetically favored, other modes of degradation can be used if needed.

Because NMD results in efficient mRNA degradation, it has been difficult to define the full extent to which this mechanism shapes the transcriptome. In addition to eliminating aberrant transcripts that contain mutations or result from processing errors, prior analyses have suggested that NMD may regulate many genes (Schweingruber et al. 2013). Lykke-Andersen et al. (2014) identified >3500 genes that produce transcripts directly sensitive to NMD. This includes most members of the SR family of splicing factors (as shown previously by Lareau et al. 2007) as well as many genes that host small nucleolar RNAs (snoRNAs) within their introns. snoRNA production is dependent on splicing of the host primary transcript followed by exonucleolytic trimming of the excised intron (Dieci et al. 2009).

snoRNAs have well-established roles in rRNA pseudouridylation, methylation, and processing, and recent work suggests that they regulate many other key cellular processes (Bratkovic and Rogelj 2014). Proposed functions include pseudouridylation of ncRNAs and mRNAs (Schwartz et al. 2014), RNA editing, and the generation of other small RNAs, including microRNAs. Furthermore, many snoRNAs are differentially expressed across development and in various diseases, including cancer, suggesting their potential role as biomarkers.

There have been suggestions that snoRNA-encoding host genes are regulated by NMD (Weischenfeldt et al. 2008), although the full extent of the regulation needed to be further developed. Each time a snoRNA is generated, a spliced RNA is also produced. Lykke-Andersen et al. (2014) found that many of these spliced RNAs are degraded by NMD, thereby allowing spliced RNA and snoRNA levels to be independently regulated despite being produced from the same gene. Consistent with this model, inhibiting NMD caused the levels of the spliced linear RNA to increase, whereas snoRNA expression did not change.

Some genes harbor multiple distinct snoRNAs, sometimes even within the same intron. Lykke-Andersen et al. (2014) found that these genes are extensively alternatively spliced and often produce spliced RNAs that are degraded by NMD. By modulating the splicing pattern used, the cell can ensure that only particular snoRNAs are generated (Fig. 1). For example, retention of an intron harboring a snoRNA prevents production of that small RNA while not affecting the processing of snoRNAs encoded in other introns. Alternatively, if the primary transcript is spliced such that two snoRNAs are present in the same intron, trimming by the exonucleases results in the production of a long ncRNA (lncRNA) that has snoRNA sequences at its mature 5′ and 3′ ends (Yin et al. 2012).

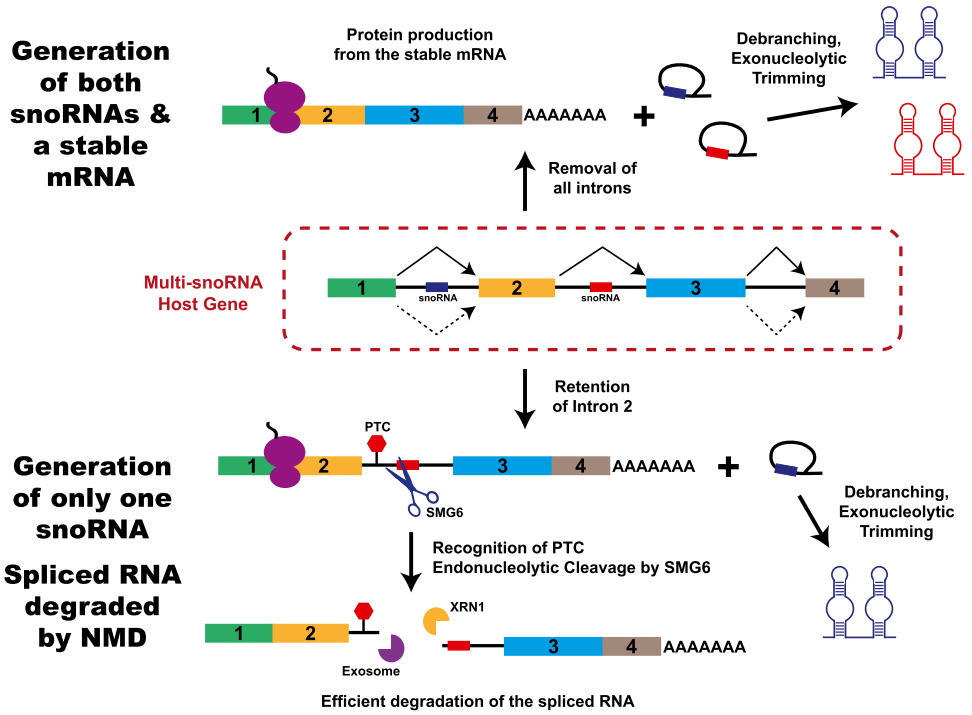

Figure 1. Alternative splicing coupled to NMD regulates the output of snoRNA host genes. (*Middle*) A model gene that hosts two snoRNAs is subjected to alternative splicing. (*Top*) When the transcript is spliced such that all introns are removed, a stable protein-coding mRNA and two snoRNA-containing intron lariats are produced. Following lariat debranching and trimming by exonucleases, the mature snoRNAs are released. (*Bottom*) If the pre-mRNA is instead spliced such that intron 2 is retained in the mature mRNA, only the snoRNA derived from the first intron is produced. Intron retention additionally causes a PTC to be present in the mRNA, which triggers NMD and endonucleolytic cleavage of the mRNA by SMG6. The two halves of the cleaved mRNA are subsequently rapidly degraded by exonucleases, such as XRN1 and the exosome. By finely regulating these alternative splicing and NMD events, the cell is able to ensure differential expression of the individual snoRNAs, spliced mRNAs, and proteins from this multifunctional gene.

In total, by coupling alternative splicing, NMD, and snoRNA biogenesis, the cell is able to generate various RNAs that have very different fates. Via this mechanism, the cell determines which RNAs are functional, and any unwanted RNA processing products that are lingering in the cytoplasm are "cleaned up." Interestingly, when one considers stress-induced activation of the IRE-1 endonuclease, tRNA processing enzymes acting on lncRNA precursors, and flaviviral RNA structures stalling exonucleases, evidence is emerging that RNA decay mechanisms besides NMD may shape the output from other multicomponent genes. Given that the 5′ ends of many RNAs do not map to conventional promoters (Kim et al. 2010), it will be informative to determine how their ends are defined and whether RNA decay factors help create novel functional transcripts.

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