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ORIGINAL RESEARCH

MicroRNA or NMD: Why Have Two RNA Silencing Systems?

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ABSTRACT

MicroRNA (miRNA)-mediated RNA silencing and nonsense-mediated decay (NMD) are two conserved RNA-level regulatory pathways. Although they are mechanically different, both can regulate target genes by RNA degradation and translational repression. Moreover, studies of individual target genes indicated that these two pathways can be involved in the same processes (e.g., development and stress responses). These facts raise an important question that whether these two systems are cooperative, interchangeable or optimal for regulation of different sorts of genes. We addressed this by comparing miRNA and NMD targets in *Arabidopsis thaliana* at the genome-wide scale. We find no more overlap in the genes targeted by both systems than expected by chance. Moreover, the sorts of genes or pathways regulated by these systems are categorically different on several cross-correlating fronts. While miRNA targets show enrichment in the process of development, metabolism and transcription, NMD targets are associated with stress responses but otherwise poorly annotated. Validated miRNA targets are more highly expressed, less variably expressed and slower evolving. These differences suggest that the modes of regulation need not be interchangeable. Instead, we suggest that miRNA genes are commonly dose-sensitive and require fine control of levels through weak pull-down by miRNAs. This is consistent with miRNA-regulated genes being more likely to be involved in protein−protein interactions. Many NMD-regulated genes, by contrast, have properties consistent with them being rapid emergency response "fire-fighter" genes. If true, the lack of annotation of NMD targets suggests that we poorly understand the emergencies plants face in the wild.

KEYWORDS: NMD; miRNA; Gene regulation; RNA degradation; Evolution

INTRODUCTION

Many eukaryotes employ two different RNA-level modes of gene expression control, microRNA (miRNA)-mediated RNA silencing and nonsense-mediated mRNA decay (NMD). miRNAs are small (about 22 nucleotides) non-coding RNAs which repress gene expression by base-paring with target mRNAs at target sites (Fig. 1) (Bartel, 2004; Hendrickson et al., 2009; Guo et al., 2010). NMD is a pathway to detect and degrade mRNA transcripts with "premature" termination codons (Fig. 1) (Chang et al., 2007; Kurihara et al., 2009; Zhang et al., 2009). NMD needs three core proteins, UPF1, UPF2 and UPF3, which can bind RNAs with recognizable premature termination codons (PTCs) (Chang et al., 2007). At first sight, the function of NMD looks like an error proofing surveillance system for mis-spliced exons or rare nonsense mutations (Losson and Lacroute, 1979). However, it also regulates expression of a subset of natural transcripts with PTC-like authentic stop codons (He et al., 2003; Rehwinkel et al., 2006; Kurihara et al., 2009; Zhang et al., 2009). Under "normal" conditions, NMD prevents abundant translation of these genes. However, when conditions change (e.g., under nutrient limitation (Mendell et al., 2004)), the NMD machinery is shut down. When this happens, normally suppressed transcripts are now free to be translated abundantly.

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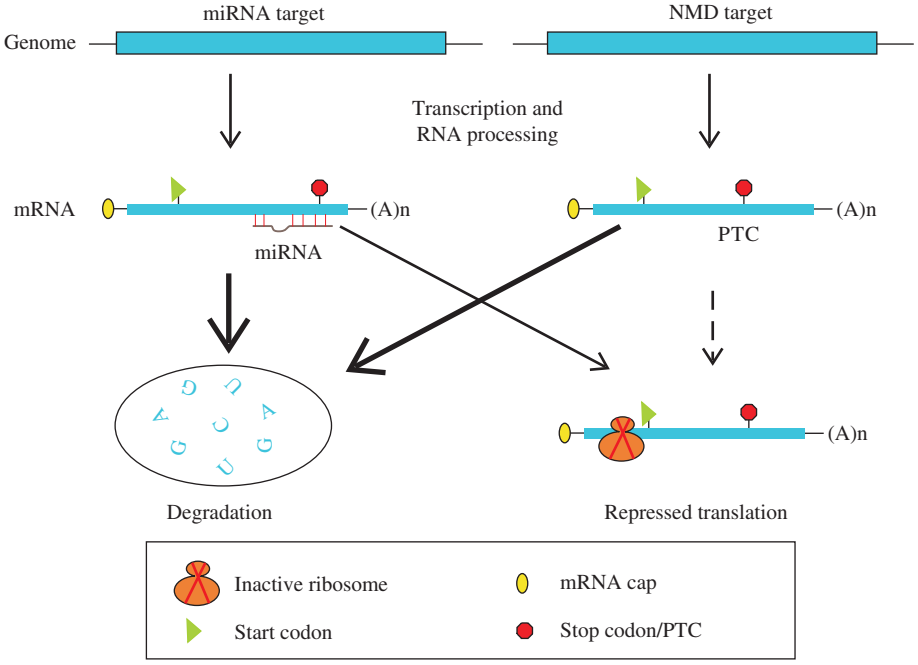

Fig. 1. The mechanisms of miRNA-mediated RNA silencing (left panel) and nonsense-mediated mRNA decay (NMD) (right panel) regulation.
In miRNA regulation, the recognition of target transcripts depends on the pairing between miRNA and target sites. In NMD targeting, a premature termination codon (PTC) is needed for target recognition. The recognition of PTC by NMD machinery is a complex process (see text). miRNA-mediated regulation mainly degrades target mRNAs (thick arrow) and marginally represses translation (thin arrow). The NMD regulation can also cause mRNA degradation but the translation repression needs further evidence (dashed line), though the NMD targets are translationally repressed (Zhang et al., 2010).

Indeed, it is striking that under nutrient limitation NMD down-regulation permits up-regulation of genes (such as those for amino acid biosynthesis) needed under nutrient limitation conditions (Mendell et al., 2004). NMD is in this instance a mode of gene regulation, but not an error proofing surveillance system. Similarly, a gene can be regulated by switching deterministically between NMD-inducing isoforms and NMD invisible isoforms (Lareau et al., 2007; Kalyna et al., 2012).

Given that both NMD and miRNA have regulatory potential with the similar regulation effect, why have two RNA-level regulatory systems? This question is all the more intriguing when one notices many similarities between the two: (1) Both regulate their targets by mRNA decay and/or repressing translation (Fig. 1) (Zhang et al., 2010; Huntzinger and Izaurralde, 2011). (2) Both are phylogenetically widespread. NMD exists in plants, animals and fungi, while miRNAs are also observed in all of them except for fungi. The precise dynamics of the two systems, however, vary across taxa. The "rules" by which cells classify a stop codon as premature show considerable variability (Conti and Izaurralde, 2005; Muhlemann, 2008; Brogna and Wen, 2009). Similarly, the degree of wobble permitted between the miRNA and the target site differs between taxa. In plants, the paired region is (nearly) completely complementary (Pasquinelli, 2012), which is more stringent than in animals. (3) Both are involved in similar cellular processes in plants. Previous studies on individual gene have shown that they are, for example, both involved in development and cellular stresses in plants. miRNA-mediated regulation can affect many developmental phenotypes, including development timing, leaf and root morphogenesis and flower development (reviewed in Rubio-Somoza and Weigel, 2011; Khraiwesh et al., 2012), while impaired NMD can result in epinastic leaves, longer seeds (Shi et al., 2012), delayed flowering time, fused flowers and lethal seedlings (Arciga-Reyes et al., 2006). Moreover, NMD is responsible for pathogen- or wounding-induced stresses (Rayson et al., 2012; Shi et al., 2012), and miRNAs were found in biotic and abiotic stress responses, including responses to bacterial pathogenesis, cold, drought, salt and so on (reviewed in Khraiwesh et al., 2012; Sunkar et al., 2012). (4) They may be mechanistically related. A recent study in HeLa cells indicated that loading of AGO2 (a key protein in miRNA targeting) or miRNA-induced silencing complex (miRISC) onto the 3′ UTR of NMD targets can abolish NMD degradation (Choe et al., 2010), suggesting that these two systems might be mechanically linked. As expected of many mRNA control systems, both involve p-bodies, foci within the cytoplasm consisting of many enzymes involved in mRNA turnover (Shyu et al., 2008). This indicates, if nothing else, that the proteins for the two systems colocalize within a cell.

These facts raise a question as to whether these two systems cooperate when they repress target genes in cells. For example, do they target the same set of genes? One could, for example, imagine that the two systems might regulate the same set of genes but under different conditions. If not, what are the determinants for the "choice" of which mechanism to employ? As both systems can repress gene expression, the "choice" of the mechanism might be historical accident. A good example consistent with this idea is that gene duplication and alternative splicing, both increasing genic proliferation,