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Invited Mini Review

Crosstalk between RNA silencing and RNA quality control in plants

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RNAs are pivotal molecules acting as messengers of genetic information and regulatory molecules for cellular development and survival. From birth to death, RNAs face constant cellular decision for the precise control of cellular function and activity. Most eukaryotic cells employ conserved machineries for RNA decay including RNA silencing and RNA quality control (RQC). In plants, RQC monitors endogenous RNAs and degrades aberrant and dysfunctional species, whereas RNA silencing promotes RNA degradation to repress the expression of selected endogenous RNAs or exogenous RNA derived from transgenes and virus. Interestingly, emerging evidences have indicated that RQC and RNA silencing interact with each by sharing target RNAs and regulatory components. Such interaction should be tightly organized for proper cellular survival. However, it is still elusive that how each machinery specifically recognizes target RNAs. In this review, we summarize recent advances on RNA silencing and RQC pathway and discuss potential mechanisms underlying the interaction between the two machineries. [BMB Reports 2023; 56(6): 321-325]

INTRODUCTION

RNAs play a pivotal role in living organisms as a converter of genetic information into functional proteins and/or a regulatory molecule to modulate a wide range of biological processes. Therefore, RNA metabolism, including transcription, modification, processing and degradation, should be precisely controlled in response to internal and external cues (1). RNAs are constantly monitored. Aberrant and/or dysfunctional RNAs are subjected to degradation by different types of RNA surveillance mechanisms such as RQC and RNA silencing (2). Exonucleolytic RQC generally occurs through bidirectional mechanisms: XRN nuclease-dependent 5′-3′ degradation and exosome-mediated 3′-5′ degradation. Deadenylation catalyzed by 3′-5′ poly(A)-specific ribonuclease (PARN) and carbon catabolites repressor 4 (CCR4) can lead to multimeric exosome complex-mediated mRNA decay, followed by 5′ decapping, which can result in 5′-3′ mRNA degradation through activities of XRN exonucleases (3). RNA silencing is responsible for the regulation of endogenous and exogenous gene expression at transcriptional and post-transcriptional levels, which is mediated by 21-24 nt small regulatory RNAs. Each machinery selectively recognizes target RNAs and promotes their degradation (2). Emerging evidences have suggested that different types of RNA surveillance mechanisms interact with each other and share target RNA substrates and regulatory components (2, 4). In this review, we discuss recent advances on RNA surveillance mechanisms and their crosstalk, focusing on the interaction between RNA silencing and RQC.

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RNA SILENCING PATHWAYS IN PLANTS

Small RNAs such as microRNAs (miRNAs) and short-interfering RNAs (siRNAs) are central contributors to RNA silencing in plants (5). Formation of double-stranded RNA is the prerequisite to turn on the RNA silencing pathway. Double stranded-RNAs originated from endogenous genes, heterochromatic regions, or exogenous genes are chopped by DICER-LIKE (DCL) enzymes to produce miRNAs or siRNAs of ~21-24 nt in length (2, 6-8). These small RNAs are incorporated into the ARGOUNAUTE complex, facilitating transcriptional or post-transcriptional levels. Generally, miRNAs of ~21 nt in length and a subset of siRNAs direct mRNA cleavage or translational inhibition to control development processes and stress responses, whereas siRNAs of ~24 nt in length repress transcriptional activation through DNA methylation and chromatin modification (2, 7-9).

One of the major functions of RNA silencing is to defense plants against viral infection and transgene introduction via post-transcriptional gene silencing (PTGS) (10, 11). A single stranded RNA from viral RNAs or transgene-derived transcripts can be recognized by RNA-DEPENDENT RNA POLYMERASE 6 (RDR6) as a substrate, which is converted into a double-stranded RNA and processed into siRNAs of ~21 nt in length (12, 13). One of the key characteristics of RDR6-dependent

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siRNA is reinforcement of the initial siRNA-mediated repressive signal. After the initial cut by siRNAs, cleaved target RNAs are subsequently processed by RDR6, resulting production of amplified siRNAs called secondary siRNAs (Fig. 1) (11). In turn, secondary siRNAs further degrade target RNAs. RDR6 also generates siRNAs from a subset of endogenous transcripts (11). The production of trans-acting siRNAs (tasiRNAs) is initiated by miRNA-directed cleavage of long non-coding TAS gene transcripts, which are further processed by RDR6-dependent siRNA biogenesis (14, 15). Unlike other siRNA species, tasiRNAs play a critical role in plant development (16, 17).

RNA QUALITY CONTROL AS A KEY MACHINERY TO ENSURE RNA FIDELITY IN PLANTS

The primary transcripts undergo multiple steps including 5′ capping, polyadenylation, splicing, and translation called RNA processing to become final and functional molecules (1). Both 5′ capping and polyadenylation prevent mRNA degradation from 5′-3′ and 3′-5′ exonucleases. Splicing removes introns from primary transcripts and ligates exons to generate mature mRNAs, which are eventually translated into functional proteins. In these processes, the RQC machinery gets rid of any unproperly processed RNAs to secure the fidelity of mRNA processing (18). In addition, eukaryotic cells monitor RNA quality during translation by surveillance mechanisms including nonsense-mediated decay (NMD), non-stop decay (NSD), and no-go decay (NGD), which can enhance RNA degradation (19). The NMD pathway facilitates RNA degradation that contains premature termination codons (20, 21). The NSD pathway targets mRNA missing translation termination code (22). The NGD pathway discriminates mRNAs with a translation elongation error represented by ribosome stalling (22).

General RNA degradation occurs bidirectionally through processes involving conserved enzymatic factors. Removal of poly(A) is accomplished by PARN and CCR4, by which RNA degradation is initiated from the unprotected 3′ ends through the exosome complex and its co-factors such as RIBOSOMAL RNA PROCESSING (RRP) proteins and the SKI complex (3, 23-25). Decapping is then processed by decapping proteins such as DECAPPING 1 (DCP1), DECAPPING 2 (DCP2), DECAPPING 3 (DCP3), and VARICOSE (VCS). Naked 5′ ends are then subjected to 5′-3′ degradation by XRN4, a predominant cytoplasmic exonuclease. In addition, XRN2 and XRN3 degrade transcripts in the nucleus (Fig. 1) (3, 26-29).

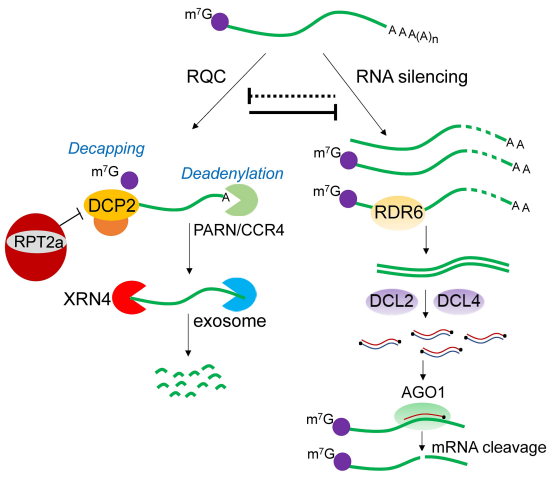

Fig. 1. RNA degradation processes controlled by the RNA quality control and the RNA silencing pathways. RNA degradation processes targeted by the RNA quality control and the RNA silencing pathways and their interaction. The RQC pathway degrades aberrant RNAs such as decapped and/or deadenylated transcripts through exonuclease activities. Exogenous and a portion of endogenous RNAs enter to the RNA silencing pathway by the production of RDR6-dependent siRNAs. The siRNAs are loaded onto AGO1 and facilitate mRNA cleavage. The RQC pathway eliminates aberrant RNAs via mRNA deadenylation and decapping-dependent bidirectional degradation. The RQC pathway suppresses RNA silencing. When the RQC pathway does not operate properly, the accumulated RNAs are served as substrates for RDR6, which turns on RNA silencing. Meanwhile, RPT2a, a subunit of 26S proteasome complex, attenuates the RQC pathway, resulting enforced RNA silencing, which suggests that the two pathways might have more dynamic bidirectional antagonistic interaction depending on the situation.

RNA SILENCING INTERACTS WITH RNA QUALITY CONTROL PATHWAY

RNA silencing and RQC were originally thought to act independently. However, emerging evidences have suggested that these two pathways can functionally interact with each other. There are solid evidences showing that a subset of components involving RNA processing and/or RQC pathways can act as suppressors of RNA silencing (30-37). In Arabidopsis, impaired decapping and deadenylation can restrict RNA silencing (33, 35, 38). Mutations in *PARN* and *CCR4a* are known to promote transgene silencing (33). In addition, decapping proteins including DCP1, DCP2, and VCS can prevent transgene PTGS. In these mutants, the production of RDR6-dependent siRNAs at the transgene locus is increased, enhancing transgene silencing (35, 38). Exonucleolytic degradation is also linked to RNA silencing (30, 39, 40). Impaired 5′-3′ or 3′-5′RNA degradation machinery can promote PTGS. In Arabidopsis, XRN proteins are responsible for 5′-3′ degradation. Functional deficiency of XRN4 can accumulate uncapped transcripts, which can trigger the production of 21 nt siRNAs and PTGS (30, 39). FIERY 1, a positive regulator of XRN proteins, can negatively regulate PTGS in an XRN-mediated manner (40). RNA surveillance accomplished by exosome-directed 3′-5′ RNA degradation through several components of exosome complex and co-factors such as RRP4, RRP41, RRP44A, and SKI3 can also suppress PTGS (34, 36, 41, 42). A recent study further supports this interaction, in which RPT2a, a subunit of 26S proteasome complex, can diminish the RQC path-

way, resulting in enhancement of transgene PTGS (Fig. 1) (37).

A large portion of studies regarding the interaction between RNA silencing and RQC have been accomplished by transgene-derived PTGS (12, 33, 36, 38, 43). However, this interaction is also effective for endogenous genes (31-35, 44). In Arabidopsis, impaired decapping machinery can enhance the production of RDR6-depedent siRNAs from hundreds of endogenous mRNAs (35). When 5′-3′ and 3′-5′ bidirectional degradation is compromised, the effect is more dramatic (44). Although a single mutant of *xrn4* or *ski2* shows a relatively mild phenotype, *xrn4 ski2* double mutants show very severe developmental retardation (44). RDR6-dependent siRNAs are generated from over 400 hundred protein coding genes. Intriguingly, morphological phenotype and siRNA production in *xrn4 ski2* can be rescued by the introduction of *rdr6* mutation, indicating that bidirectional RNA decay pathways play a critical role in preventing the trigger of RNA silencing.

PATH TO RNA SILENCING

RNA silencing and RQC compete for the same RNA substrates. How does a cell determine RNA's fate: its entry to RNA silencing or the RQC pathway? Although the exact mechanism remains elusive, emerging research studies have accumulated important clues to answer this question. Studies on transgene PTGS have raised a possibility that the expression level of transgene is correlated with the entry to RNA silencing (45). For example, ectopic expression of transgene showed a high rate of induction of RNA silencing, whereas ectopically expressed transgenes did not always undergo PTGS (45). Furthermore, expression levels of endogenous protein-coding mRNAs are not correlated to the production of RDR6-depedent siRNAs (35). Therefore, RNA quantity is not a sufficient factor for the entry of RNA silencing. In another aspect, RNA quality or characteristic is also important. Decapped and improperly terminated RNAs are likely to serve as substrates for RDR6, which can trigger RNA silencing (35, 38). In addition, key components of NMD have been isolated as suppressors of RNA silencing (33, 43). Mutations on *UP FRAMESHIFT 1* (*UPF1*) and *UP FRAMESHIFT 3* (*UPF3*) can promote transgene PTGS, indicating that RNA quality is somehow linked to the evocation of RNA silencing. Besides, recent studies have suggested that non-canonical RNA modification such as NAD^+ capping can promote RDR6-dependent PTGS (46, 47). These findings indicate that RNA quantity and quality monitored by a cell could be criteria to provoke RNA silencing. Accumulated evidences have indicated that the fate of RNAs to RNA silencing and/or the RQC pathway is systematically regulated. However, further investigation is needed to understand mechanisms underlying it.

CONCLUSION

A eukaryotic cell employs RNA silencing and/or RQC to maintain RNA integrity. The RQC pathway eliminates aberrant or dysfunctional RNAs to prevent production of toxic proteins. RNA silencing represses target RNAs, which is critical for plant development and defense. It is evident that RNA silencing and RQC pathways interact with each other. If the interaction is not properly regulated, RNA quality will become disordered. When the RQC machinery is impaired, aberrant and dysfunctional RNAs will become over-accumulated, which can served as templates for RDR6 and amplify repressive signals for unwanted endogenous RNAs. Therefore, a plant cell should be able to monitor and decide target RNA's destiny into either RQC or RNA silencing. Great efforts have been made to dissect the molecular mechanism underlying this interaction in Arabidopsis. Our understanding is not complete yet.

The RQC pathway has been thought to play an important role in deciding whether a shared target RNA should enter into RQC or RNA silencing because the accessibility of RDR6 to the target RNA depends on the activity of RQC. A recent study has shown that 26S proteasome-mediated repression of the RQC pathway can promote transgene PTGS in Arabidopsis (37) . This result suggests that the cellular system might adjust the interaction between RNA silencing and RQC according to internal/external cues, which needs further investigation.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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