



Emerging value of the viroid model in molecular biology and beyond

Junfei Ma¹, Shachinthaka D. Dissanayaka Mudiyansele¹, Ying Wang^{*}

Department of Biological Sciences, Mississippi State University, MS 39762, USA

ARTICLE INFO

Keywords:

Viroid
RNA-templated transcription
Pol II
RNA trafficking
Plant innate immunity

ABSTRACT

Viroids are single-stranded circular noncoding RNAs that infect plants. Research in the past five decades has deciphered the viroid genome structures, viroid replication cycles, numerous host factors for viroid infection, viroid motifs for intracellular and intercellular trafficking, interactions with host defense machinery, etc. In this review, we mainly focus on some significant questions that remain to be tackled, centered around (1) how the RNA polymerase II machinery performs transcription on RNA templates of nuclear-replicating viroids, (2) how viroid RNAs coordinate multiple structural elements for diverse functions, and (3) how viroid RNAs activate plant immunity. Research on viroids has led to seminal discoveries in biology, and we expect the research directions outlined in this review to continue providing key knowledge inspiring other areas of biology.

1. Introduction

The first viroid, potato spindle tuber viroid (PSTVd), was discovered and named in 1971 (Diener, 1971; Diener, 2018). Since then, about 40 viroids have been reported and categorized into two families, *Pospiviroidae* and *Avsunviroidae* (Di Serio et al., 2018; Di Serio et al., 2021). All the known viroids are circular noncoding RNAs that infect plants (Flores et al., 2016; Navarro et al., 2021; Wang, 2021). Members of the family *Pospiviroidae* possess rod-shaped genome structures and replicate in the nucleus. Transcription from their circular genome to multimeric (-) intermediates is known to harness DNA-dependent RNA polymerase II (Pol II) (Mühlbach and Sänger, 1979; Rackwitz et al., 1981). An RNA-specific transcription factor (TFIIIA-7ZF) is critical for redirecting Pol II to use the RNA genome of PSTVd, a representative species of *Pospiviroidae* (Wang et al., 2016). Viroids of the family *Avsunviroidae* all replicate in chloroplasts and adapt a highly branched portion in their RNA genomes (Flores et al., 2016; Navarro et al., 2021; Wang, 2021). They rely on nuclear-encoded polymerase (NEP) for RNA-templated transcription (Navarro et al., 2000).

Viroids can spread systematically after replication in compatible host plants. Studies on PSTVd structural motifs have identified a series of RNA 3-dimensional (3D) motifs regulating RNA movement across various cellular boundaries (Wang et al., 2018; Zhong et al., 2008). A recent finding also identifies a critical, and likely conserved, RNA 3D motif regulating the nuclear import of viroids of the family *Pospiviroidae* (Ma et al., 2021). These discoveries support a model that viroids harness

their RNA 3D motifs for intracellular, intercellular and systemic trafficking.

While numerous reports described symptomless viroid infection in some hosts (Daros, 2016; Li et al., 2021a; Verhoeven et al., 2013), many viroids are well-known pathogens to economically important crops (Flores et al., 2020; Flores et al., 2016; Navarro et al., 2012). Recent comprehensive transcriptome analyses from multiple groups showed that viroid infection can elicit host immune responses (Kappagant et al., 2017; Thibaut and Claude, 2018; Zheng et al., 2017), in addition to the well-known viroid interaction with host RNA silencing machinery (Ding, 2009; Navarro et al., 2021). These insights provide a framework to understand the pathogenesis that is caused by foreign RNA alone.

In this review, we mainly discuss some of the emerging questions in plant-viroid interactions and provide testable hypotheses for future investigations, particularly revolving around the composition and mechanism of Pol II machinery on viroid RNA templates, the functional RNA 3D motifs in regulating RNA trafficking in plants, as well as plant defense against viroids from the innate immunity perspective.

2. Viroid RNA-templated transcription in the nucleus

Mounting evidence supports that viroids of the family *Pospiviroidae* use host Pol II to catalyze the transcription from circular genome to linear multimeric (-) intermediates (Mühlbach and Sänger, 1979; Rackwitz et al., 1981). In contrast to Pol I, Pol III and NEP, Pol II has a specialized carboxy-terminal domain (CTD) that supports

^{*} Correspondence author

E-mail address: wang@biology.msstate.edu (Y. Wang).

¹ These authors contributed equally to this work.

co-transcriptional regulation (Bentley, 2014). While Pol II-catalyzed cellular mRNA synthesis is proceeding, a G cap is attached to nascent transcripts and introns are removed. This is feasible because the conserved “YSPTSPS” heptapeptide repeats in the Pol II CTD domain can recruit distinct factors based on the different phosphorylation statuses (Corden et al., 1985; Egloff et al., 2012). Viroids clearly do not exploit the whole set of co-transcription machinery for their own replication, therefore providing opportunities to understand the regulation of transcription on RNA templates with limited co-transcriptional regulation.

2.1. Viroid RNAs do not possess a G cap

The G cap attachment can be a simultaneous process during the transcription of many cellular mRNAs (Bentley, 2014). When nascent transcripts reach a length of ~20–30 nt, these short transcripts become capped near the RNA exit channel of the Pol II complex (Rasmussen and Lis, 1993). The phosphorylation of Ser5 in the heptapeptide repeats is critical to recruiting nuclear capping enzyme and guanine-7-methyltransferase for co-transcriptional capping (Cho et al., 1997; Ho and Shuman, 1999; McCracken et al., 1997).

The capping process coincides with the promoter-proximal pause during the transcription of many metazoan genes (Adelman and Lis, 2012; Lenasi et al., 2011; Rasmussen and Lis, 1993). During this transcriptional pause, the nuclear capping enzyme interacts with the elongation factor SPT4/5. Phosphorylation of SPT4/5 releases pausing to resume productive elongation (Mandal et al., 2004; Pei and Shuman, 2002; Wen and Shatkin, 1999). The promoter-proximal pause serves as a checkpoint for coupling the G cap addition and elongation as well as allows the preparation of permissive DNA templates.

To date, there is no evidence showing that viroid transcripts possess any G cap. In addition, transcriptional pausing has not been clearly demonstrated in the RNA-templated transcription either. These observations prompt the question of whether the phosphorylation codes of the Pol II CTD domain are necessary for RNA-templated transcription. Notably, Pol II-dependent RNA-templated transcription of B2 ncRNA and the human hepatitis delta virus (HDV) genomic/antigenomic RNAs do not possess G cap either (Taylor, 2015; Wagner et al., 2013). The HDAg mRNA from HDV with a defined G cap accounts for an extremely low percentage of total HDV RNAs (Gudima et al., 2000), but it is unclear whether the G cap in HDAg mRNA is added through co-transcriptional activity or other activities such as through cytosolic G cap enzyme. Therefore, the replication of viroids and HDV genomes relies on Pol II but has no known co-transcriptional regulation. Future studies on viroid replication may provide insights into the composition and *modus operandi* of Pol II complex on RNA templates.

2.2. Splicing and nuclear export

Intron splicing can occur co-transcriptionally or post-transcriptionally (Aslanzadeh et al., 2018; Bentley, 2014; Drexler et al., 2020). The assembly of spliceosomes on nascent transcripts regulates the co-transcriptional splicing (Herzel et al., 2017). Importantly, splicing and nuclear export are coupled via the TREX complex, which is conserved in metazoans and contains both splicing factors and nuclear export factors (Strasser et al., 2002). Hence, splicing is a necessary step for the nuclear export of most cellular mRNAs.

Nuclear-replicating viroids do not harbor any intron, despite their dependence on Pol II for replication. Moreover, unlike the cellular mRNAs that are transported to the cytoplasm directly from their synthesis sites in the nucleoplasm, PSTVd appears to be transported into the nucleolus from the production site before nuclear export (Qi and Ding, 2003). Therefore, viroids probably harness another route for nuclear export. One hypothesis is that viroids can use the 5S rRNA nuclear export pathway through transcription factor IIIA (the full-length protein with nine zinc fingers; TFIIA-9ZF) (Pieler and Rudt, 1997). Evidence showed that TFIIA-9ZF interacts with PSTVd *in vivo*, but the functional

significance of this interaction awaits future investigation (Wang et al., 2016). Alternatively, viroid nuclear export may rely on another pathway yet-to-be-elucidated.

2.3. Transcription termination

It is well accepted that cleavage at the poly(A) site (i.e., AAUAAA) leads to the addition of the poly(A) tail to mRNAs and the exonuclease-involved transcription termination (Proudfoot and Brownlee, 1976; Shi and Manley, 2015; Tian and Graber, 2012). However, the transcripts using the AAUAAA signal for termination only account for ~10% in plants (Wu et al., 2011). Recently, a specific histone deacetylase (HDA6) has been shown to regulate the usage of poly(A) sites other than AAUAAA in *Arabidopsis*, possibly through modifying the epigenetic hallmarks on DNA templates surrounding the AAUAAA sites (Lin et al., 2020). This observation indicates that transcriptional termination may be regulated via multiple means in plants.

The regulation of Pol II termination on RNA templates has not been fully understood, except for the HDV HDAG gene that contains a functional AAUAAA element (Hsieh et al., 1990). Early studies found that the replication intermediates from circular (+) viroid templates have relatively unique size ranges based on viroid species. For example, PSTVd (-) intermediates can be tetramers (Branch et al., 1981; Spiesmacher et al., 1983) or hexamers (Muhlbach et al., 1983); citrus exocortis viroid (CEVd) (-) intermediates are roughly 6–8 times monomer in size (Hutchins et al., 1985); and hop stunt viroid (HSVd) (-) intermediates are dominantly dimers or tetramers (Ishikawa et al., 1984). While it is intuitive to consider the template run-off theme that Pol II leaves templates after several rounds of transcription on circular RNA templates, other possibilities involving regulatory factors cannot be ruled out. The relatively species-specific consistency in the lengths of (-) intermediates implies the existence of regulation over transcription termination when Pol II uses viroid genomic RNA as templates for transcription. Interestingly, reports showed that HSVd interacts with HDA6 directly and affects its cellular function (Castellano et al., 2016). Whether HDA6 is involved in regulating transcription termination on RNA templates may be clarified in future investigations.

3. RNA structure-mediated viroid trafficking

Due to their noncoding nature, viroids rely on their RNA structures to harness host factors for infection. Genome-wide analysis on PSTVd local motifs found that many RNA loop structures are critical for either replication or systemic trafficking (Zhong et al., 2008). Detailed analyses have uncovered the function of those trafficking-related loops in regulating nuclear import and spreading across various cellular boundaries.

3.1. RNA structure-mediated nuclear import

Although it is well known that members of *Pospiviroidae* enter the nucleus for replication (Ding, 2009), the regulatory mechanism for nuclear imports remains unclear. Recently, we identified a critical C-loop for viroid nuclear imports. C-loop is the binding site for Virp1, a known host factor for viroid infection (Martínez de Alba et al., 2003). Previous studies suggest that Virp1 recognizes at least one of the two RY motifs in PSTVd (Gozmanova et al., 2003). RY motif appears to be conserved in members of *Pospiviroidae*. We found the PSTVd C-loop partially overlaps with the RY motif closer to the right terminus (Ma et al., 2021). Point mutations in PSTVd C-loop strongly impair PSTVd infectivity, nuclear accumulation, and interaction with Virp1. We also found a C-loop in HSVd, which is not overlapping with the described RY motif therein. Point mutations in HSVd C-loop also strongly impair HSVd infectivity and interaction with Virp1, supporting that C-loop is the *bona fide* binding site of Virp1 (Ma et al., 2021). C-loop can be found in nearly all, except one, formal members of *Pospiviroidae* (Ma et al., 2021) and even

in a satellite RNA of cucumber mosaic virus that relies on Virp1 for nuclear import (Chaturvedi et al., 2014). Altogether, C-loop is probably a conserved signal regulating the nuclear imports of plant subviral RNAs.

3.2. RNA structure-mediated systemic infection

In a simplified view, viroids need to move from leaf epidermis, through palisade mesophyll and spongy mesophyll, to cross bundle sheath and enter phloem for systemic trafficking. Viroids will also need to cross bundle sheath and invade mesophyll and epidermis in systemic leaves (Wang and Ding, 2010). Strikingly, PSTVd possesses at least one RNA motif regulating the trafficking across most of these tissues.

The right terminal loop is critical for movement from epidermis to mesophyll (Wu et al., 2019). Loops 6 and 19 both regulate trafficking from palisade mesophyll to spongy mesophyll (Jiang et al., 2017; Takeda et al., 2011). Loop 7 dictates the phloem entry from bundle sheath (Zhong et al., 2007). A bipartite motif controls phloem exiting to bundle sheath in systemic leaves (Qi et al., 2004). An emerging model from these data outlines that distinct RNA structural motifs contain the necessary information for crossing various checkpoints between diverse tissue types.

3.3. Organizing multiple functional elements in one RNA

The presence of the bipartite motif regulating the bundle sheath exit of PSTVd is intriguing (Qi et al., 2004). It suggests that viroid genomic RNAs will alter the rod-shaped structures to achieve certain functions in plants. Perhaps an alternative possibility to explain the functional redundancy of loop 6 and loop 19 is that they form some sort of bipartite element as well. Another intriguing question is how viroids organize so many elements for diverse functions without interfering with each other. For instance, how do viroids coordinate (1) nuclear import and nuclear export as well as (2) nuclear import and intercellular trafficking? Investigations on these questions may help elucidate the coordination of diverse functions of a single RNA molecule through organizing its structure in the dynamic cellular environment, which may advance the understanding of RNA structure-function relationships to a deeper level.

4. Viroid and host defense

It becomes clear that plant defense against viroid infection relies on both RNA silencing and innate immunity (Flores et al., 2020; Navarro et al., 2021). Viroid infection generates small RNAs (sRNAs) ranging from 20–24 nt in size. These viroid-derived sRNAs (vd-sRNAs) likely play an inhibitory role in viroid replication. Viroid infection also triggers host immune responses that reprogram host gene expression to activate ROS signaling, cell wall fortification, and hormonal pathways related to defense. In general, the activation of immune responses often has impacts on plant signaling and metabolism that lead to cytopathic effects and alterations in morphology.

4.1. Viroid interaction with host RNA silencing machinery

Based on the current model, the replication of viroids will generate double-stranded intermediates that will be cleaved by various Dicer-like proteins (DCLs) in plants (Ding, 2010). Noteworthy is that the PSTVd RNA genome without replication can also be a target of DCLs purified from plants (Itaya et al., 2007). In general, DCL2 and DCL3 synergistically suppress PSTVd infection, whereas DCL4 somehow positively regulates PSTVd replication in *Nicotiana benthamiana* (Dadami et al., 2013; Katsarou et al., 2016). Viroid-derived sRNAs can be loaded into Argonaute proteins (AGOs) for function (Itaya et al., 2007; Minoia et al., 2014). Specifically, *Agrobacterium tubefaciens*-mediated transient expression of *Arabidopsis* AGOs followed by RNA-immunoprecipitation revealed that plant AGO1, AGO2, AGO3, AGO4, AGO5, AGO8, and

AGO9 all have the ability to recruit vd-sRNAs. AGO1, AGO2, and AGO3 favor the binding of 21- and 22-nt vd-sRNAs, while AGO4, AGO5, and AGO9 enrich a good portion of 24-nt vd-sRNAs. Interestingly, ectopic expression of AGO1, AGO2, AGO4, or AGO5 attenuated PSTVd titers in infected *N. benthamiana*, supporting their roles in plant-viroid interactions (Minoia et al., 2014).

RNA-dependent RNA polymerase 6 (RDR6), a critical player in the RNA silencing pathway, plays a role in preventing PSTVd from invading shoot apical meristem in host plants (i.e., *N. benthamiana* and tomato) (Di Serio et al., 2010; Naoi et al., 2020). Importantly, RDR6 also modulates HSVd-triggered pathogenicity in plants (Gomez et al., 2008). The detailed mechanism underlying the role of RDR6 in controlling PSTVd tissue tropism and pathogenicity remains to be determined. A recent report also shows that perturbing the expression of RDR1 affects PSTVd infectivity (Li et al., 2021b). However, RDR1 expression remains unchanged in PSTVd- and CEVd-infected plants based on reported RNA-Seq data (Thibaut and Claude, 2018; Zheng et al., 2017), rendering it questionable whether RDR1 is a *bona fide* defense gene against viroid.

4.2. Viroid interaction with plant innate immunity

Plants generally deploy a two-layer immunity defending various pathogens, namely pathogen-associated molecular patterns (PAMPs)-triggered immunity (PTI) and effector-triggered immunity (ETI) (Jones and Dangl, 2006). PTI functions mainly at cell surface, whereas ETI largely occurs within cells. The orchestrated PTI and ETI activities are essential for plant survival.

The framework that the presence of viroids can trigger host immune responses has been established recently (Kappagant et al., 2017; Thibaut and Claude, 2018; Zheng et al., 2017). How can plant cells sense the presence of foreign RNAs (i.e., viroids) and activate the innate immune system remains obscure. Previously it was thought that plants utilize PKV (protein kinase, viroid-induced), a double-stranded-RNA-binding protein kinase, to sense viroid RNAs and triggers defense signaling (Hammond and Zhao, 2000; Hammond and Zhao, 2009). However, this PKV appears to be a pseudogene based on comprehensive RNA-Seq analyses (Thibaut and Claude, 2018; Zheng et al., 2017). Viroids may not trigger PTI response because they enter host cells mainly through wounding or, to a lesser extent based on current knowledge, insect vectors (Matsushita et al., 2018). Within the infected plants, viroids move through plasmodesmata (Ding et al., 1997). Therefore, viroids are rarely present on cell surface to elicit PTI. Whether viroids can activate ETI is a puzzle because an R gene that can specifically sense viroids has not been found. If viroid does not trigger ETI response, then the immune responses elicited by viroids might be attributable to damage-associated molecular pattern-triggered immunity that was activated by the emission of cell damage related signal molecules yet-to-be-identified (Boutrot and Zipfel, 2017; Erb and Reymond, 2019; Hou et al., 2019). Interestingly, a recent study showed that PSTVd replication can lead to up-regulation of miR398-regulated production of reactive oxygen species (Fujibayashi et al., 2021; Suzuki et al., 2019), which may link the activity of RNA silencing and innate immunity in defending viroids. It will be critical to elucidate the detailed mechanism underlying the regulation of miR398 and the detailed events along this regulatory cascade in viroid-infected plants. Undoubtedly, the efforts to understand the viroid-triggered immune response will shed light on the mechanism for plants to perceive the presence of foreign RNAs.

5. Concluding remarks

As a peculiar group of subviral agents, viroids can serve as a productive model to tackle basic questions in molecular biology and beyond. PSTVd is the first known natural RNA redirecting a DNA-dependent RNA polymerase (DdRP) for transcription. To date, intrinsic RNA-dependent RNA polymerase (RdRP) activity has been found in many DdRPs (Dissanayaka Mudiyansele et al., 2018). These

observations imply a missing link in the molecular evolution of transcription, where RNA templates emerged first before DNA templates (Lehmann et al., 2007). The viroid model can help dissect the needed factors for redirecting DdRPs to use RNA templates as well as distinguish the differences in regulating efficient transcription on DNA and RNA templates. This line of research is highly related to studies on HDV, which also relies on the RdRP activity of host DdRPs (Taylor, 2015). Viroids also serve as a productive model to delineate RNA structure-function relationships. The function of many PSTVd RNA motifs has been illustrated, and some of them have been studied in detail in terms of structures and cognate factors. It is particularly interesting to expand similar analyses to other viroids, and even RNA viruses, to establish a general view of RNA motif organizations for effective infection. Last but not the least, it is an exciting area to study the interaction between viroids and host innate immunity. RNAs represent a universal component in all pathogens, yet their direct interactions with plant innate immunity are often overlooked. The viroid model can be useful to advance our understanding of plant innate immunity in terms of sensing foreign RNAs, including but not limited to viruses and viroids.

CRedit authorship contribution statement

Junfei Ma: Writing – review & editing. **Shachinthaka D. Dissanayaka Mudiyansele:** Writing – review & editing. **Ying Wang:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgements

This review is in dedication to the memory of late professor Dr. Ricardo Flores, a true leader and a caring colleague in the field of viroid research. This work was supported by the National Institutes of Health [1R15GM135893 to YW] and the US National Science Foundation [MCB-1906060 and MCB-2145967 to YW]. We apologize to colleagues whose work was not cited in this review due to the page limit.

References

- Adelman, K., Lis, J.T., 2012. Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. *Nat. Rev. Genet.* 13 (10), 720–731.
- Aslanzadeh, V., Huang, Y., Sanguinetti, G., Beggs, J.D., 2018. Transcription rate strongly affects splicing fidelity and cotranscriptionality in budding yeast. *Genome Res.* 28 (2), 203–213.
- Bentley, D.L., 2014. Coupling mRNA processing with transcription in time and space. *Nat. Rev. Genet.* 15 (3), 163–175.
- Boutrot, F., Zipfel, C., 2017. Function, discovery, and exploitation of plant pattern recognition receptors for broad-spectrum disease resistance. *Annu. Rev. Phytopathol.* 55, 257–286.
- Branch, A.D., Robertson, H.D., Dickson, E., 1981. Longer-than-unit-length viroid minus strands are present in RNA from infected plants. *Proc. Natl. Acad. Sci. USA* 78 (10), 6381–6385.
- Castellano, M., Pallas, V., Gomez, G., 2016. A pathogenic long noncoding RNA redesigns the epigenetic landscape of the infected cells by subverting host histone deacetylase 6 activity. *New Phytol.* 211 (4), 1311–1322.
- Chaturvedi, S., Kalantidis, K., Rao, A.L., 2014. A bromodomain-containing host protein mediates the nuclear importation of a satellite RNA of cucumber mosaic virus. *J. Virol.* 88 (4), 1890–1896.
- Cho, E.J., Takagi, T., Moore, C.R., Buratowski, S., 1997. mRNA capping enzyme is recruited to the transcription complex by phosphorylation of the RNA polymerase II carboxy-terminal domain. *Genes Dev.* 11 (24), 3319–3326.
- Corden, J.L., Cadena, D.L., Ahearn, J.M., Dahmus, M.E., 1985. A unique structure at the carboxyl terminus of the largest subunit of eukaryotic RNA polymerase II. *Proc. Natl. Acad. Sci. USA* 82 (23), 7934–7938.
- Dadami, E., Boutla, A., Vrettos, N., Tzortzakaki, S., Karakasilioti, I., Kalantidis, K., 2013. DICER-LIKE 4 but not DICER-LIKE 2 may have a positive effect on potato spindle tuber viroid accumulation in *Nicotiana benthamiana*. *Mol. Plant* 6 (1), 232–234.
- Daros, J.A., 2016. Eggplant latent viroid: a friendly experimental system in the family *avsunviroidae*. *Mol. Plant Pathol.* 17 (8), 1170–1177.
- Di Serio, F., Li, S.F., Matousek, J., Owens, R.A., Pallas, V., Randles, J.W., Sano, T., Verhoeven, J.T.J., Vidalakis, G., Flores, R., ICTV Report, C., 2018. ICTV virus taxonomy profile: *avsunviroidae*. *J. Gen. Virol.* 99 (5), 611–612.
- Di Serio, F., Martinez de Alba, A.E., Navarro, B., Gisela, A., Flores, R., 2010. RNA-dependent RNA polymerase 6 delays accumulation and precludes meristem invasion of a viroid that replicates in the nucleus. *J. Virol.* 84 (5), 2477–2489.
- Di Serio, F., Owens, R.A., Li, S.F., Matousek, J., Pallas, V., Randles, J.W., Sano, T., Verhoeven, J.T.J., Vidalakis, G., Flores, R., ICTV Report, C., 2021. ICTV virus taxonomy profile: *pospiroviroidae*. *J. Gen. Virol.* 102 (2), 001543.
- Diener, T.O., 1971. Potato spindle tuber "virus". IV. A replicating, low molecular weight RNA. *Virology* 45 (2), 411–428.
- Diener, T.O., 2018. Of viroids and prions. *Viruses* 10 (12), 663.
- Ding, B., 2009. The biology of viroid-host interactions. *Annu. Rev. Phytopathol.* 47, 105–131.
- Ding, B., Kwon, M.O., Hammond, R., Owens, R., 1997. Cell-to-cell movement of potato spindle tuber viroid. *Plant J.* 12 (4), 931–936.
- Ding, S.W., 2010. RNA-based antiviral immunity. *Nat. Rev. Immunol.* 10 (9), 632–644.
- Dissanayaka Mudiyansele, S.D., Qu, J., Tian, N., Jiang, J., Wang, Y., 2018. Potato spindle tuber viroid RNA-templated transcription: factors and regulation. *Viruses* 10 (9), 503.
- Drexler, H.L., Choquet, K., Churchman, L.S., 2020. Splicing kinetics and coordination revealed by direct nascent RNA sequencing through nanopores. *Mol. Cell* 77 (5), 985–998 e988.
- Egloff, S., Dienstbier, M., Murphy, S., 2012. Updating the RNA polymerase CTD code: adding gene-specific layers. *Trends Genet.* 28 (7), 333–341.
- Erb, M., Reymond, P., 2019. Molecular interactions between plants and insect herbivores. *Annu. Rev. Plant Biol.* 70, 527–557.
- Flores, R., Navarro, B., Delgado, S., Serra, P., Di Serio, F., 2020. Viroid pathogenesis: a critical appraisal of the role of RNA silencing in triggering the initial molecular lesion. *FEMS Microbiol. Rev.* 44 (3), 386–398.
- Flores, R., Owens, R.A., Taylor, J., 2016. Pathogenesis by subviral agents: viroids and hepatitis delta virus. *Curr. Opin. Virol.* 17, 87–94.
- Fujibayashi, M., Suzuki, T., Sano, T., 2021. Mechanism underlying potato spindle tuber viroid affecting tomato (*Solanum lycopersicum*): loss of control over reactive oxygen species production. *J. Gen. Plant Pathol.* 87 (4), 226–235.
- Gomez, G., Martinez, G., Pallas, V., 2008. Viroid-induced symptoms in *Nicotiana benthamiana* plants are dependent on RDR6 activity. *Plant Physiol.* 148 (1), 414–423.
- Gozmanova, M., Denti, M.A., Minkov, I.N., Tsagris, M., Tabler, M., 2003. Characterization of the RNA motif responsible for the specific interaction of potato spindle tuber viroid RNA (PSTVd) and the tomato protein Virp1. *Nucleic Acids Res.* 31 (19), 5534–5543.
- Gudima, S., Wu, S.Y., Chiang, C.M., Moraleta, G., Taylor, J., 2000. Origin of hepatitis delta virus mRNA. *J. Virol.* 74 (16), 7204–7210.
- Hammond, R.W., Zhao, Y., 2000. Characterization of a tomato protein kinase gene induced by infection by Potato spindle tuber viroid. *Mol. Plant Microbe Interact.* 13 (9), 903–910.
- Hammond, R.W., Zhao, Y., 2009. Modification of tobacco plant development by sense and antisense expression of the tomato viroid-induced AGC VIIIA protein kinase PKV suggests involvement in gibberellin signaling. *BMC Plant Biol.* 9, 108.
- Herzel, L., Ottoz, D.S.M., Alpert, T., Neugebauer, K.M., 2017. Splicing and transcription touch base: co-transcriptional spliceosome assembly and function. *Nat. Rev. Mol. Cell Biol.* 18 (10), 637–650.
- Ho, C.K., Shuman, S., 1999. Distinct roles for CTD Ser-2 and Ser-5 phosphorylation in the recruitment and allosteric activation of mammalian mRNA capping enzyme. *Mol. Cell* 3 (3), 405–411.
- Hou, S., Liu, Z., Shen, H., Wu, D., 2019. Damage-associated molecular pattern-triggered immunity in plants. *Front. Plant Sci.* 10, 646.
- Hsieh, S.Y., Chao, M., Coates, L., Taylor, J., 1990. Hepatitis delta virus genome replication: a polyadenylated mRNA for delta antigen. *J. Virol.* 64 (7), 3192–3198.
- Hutchins, C.J., Keese, P., Visvader, J.E., Rathjen, P.D., McInnes, J.L., Symons, R.H., 1985. Comparison of multimeric plus and minus forms of viroids and virusoids. *Plant Mol. Biol.* 4 (5), 293–304.
- Ishikawa, M., Meshi, T., Ohno, T., Okada, Y., Sano, T., Ueda, I., Shikata, E., 1984. A revised replication cycle for viroids: the role of longer than unit length RNA in viroid replication. *Mol. Gen. Genet.* 196 (3), 421–428.
- Itaya, A., Zhong, X., Bundschuh, R., Qi, Y., Wang, Y., Takeda, R., Harris, A., Molina, C., Nelson, R., Ding, B., 2007. A structured viroid RNA serves as a substrate for dicer-like cleavage to produce biologically active small RNAs but is resistant to RNA-induced silencing complex-mediated degradation. *J. Virol.* 81 (6), 2980–2994.
- Jiang, D., Wang, M., Li, S., 2017. Functional analysis of a viroid RNA motif mediating cell-to-cell movement in *Nicotiana benthamiana*. *J. Gen. Virol.* 98 (1), 121–125.
- Jones, J.D., Dangl, J.L., 2006. The plant immune system. *Nature* 444 (7117), 323–329.
- Kappagant, M., Bullock, J.M., Nelson, M.E., Eastwell, K.C., 2017. Hop stunt viroid: effect on host (*Humulus lupulus*) transcriptome and its interactions with hop powdery mildew (*Podosphaera macularis*). *Mol. Plant Microbe Interact.* 30 (10), 842–851.
- Katsarou, K., Mavrothalassiti, E., Dermauw, W., Van Leeuwen, T., Kalantidis, K., 2016. Combined activity of DCL2 and DCL3 is crucial in the defense against potato spindle tuber viroid. *PLoS Pathog.* 12 (10), e1005936.
- Lehmann, E., Brueckner, F., Cramer, P., 2007. Molecular basis of RNA-dependent RNA polymerase II activity. *Nature* 450 (7168), 445–449.
- Lenasi, T., Peterlin, B.M., Barboric, M., 2011. Cap-binding protein complex links pre-mRNA capping to transcription elongation and alternative splicing through positive transcription elongation factor b (p-TEFb). *J. Biol. Chem.* 286 (26), 22758–22768.

- Li, S., Wu, Z., Zhou, Y., Dong, Z., Fei, X., Zhou, C., Li, S., 2021a. Changes in metabolism modulate induced by viroid infection in the orchid *dendrobium officinale*. *Virus Res.* 308, 198626.
- Li, S., Zhang, Z., Zhou, C., Li, S., 2021b. RNA-dependent RNA polymerase 1 delays the accumulation of viroids in infected plants. *Mol. Plant Pathol.* 22 (10), 1195–1208.
- Lin, J., Hung, F., Ye, C., Hong, L., Shih, Y., Wu, K., Li, Q.Q., 2020. HDA6-dependent histone deacetylation regulates mRNA polyadenylation in arabidopsis. *Genome Res.* 30 (10), 1407–1417.
- Ma, J., Dissanayaka Mudiyanse, S.D., Park, W.J., Wang, M., Takeda, R., Liu, B., Wang, Y., 2021. A nuclear import pathway exploited by viroid RNAs. *bioRxiv*, 2012 (2002), 470923, 2021.
- Mandal, S.S., Chu, C., Wada, T., Handa, H., Shatkin, A.J., Reinberg, D., 2004. Functional interactions of RNA-capping enzyme with factors that positively and negatively regulate promoter escape by RNA polymerase II. *Proc. Natl. Acad. Sci. USA* 101 (20), 7572–7577.
- Martínez de Alba, A.E., Sägeser, R., Tabler, M., Tsagris, M., 2003. A bromodomain-containing protein from tomato specifically binds potato spindle tuber viroid RNA *in vitro* and *in vivo*. *J. Virol.* 77 (17), 9685–9694.
- Matsushita, Y., Yanagisawa, H., Sano, T., 2018. Vertical and horizontal transmission of pospiviroids. *Viruses* 10 (12), 706.
- McCracken, S., Fong, N., Rosonina, E., Yankulov, K., Brothers, G., Siderovski, D., Hessel, A., Foster, S., Shuman, S., Bentley, D.L., 1997. 5'-Capping enzymes are targeted to pre-mRNA by binding to the phosphorylated carboxy-terminal domain of RNA polymerase II. *Genes Dev.* 11 (24), 3306–3318.
- Minoia, S., Carbonell, A., Di Serio, F., Gisel, A., Carrington, J.C., Navarro, B., Flores, R., 2014. Specific argonautes selectively bind small RNAs derived from potato spindle tuber viroid and attenuate viroid accumulation *in vivo*. *J. Virol.* 88 (20), 11933–11945.
- Mühlbach, H.P., Faustmann, O., Sanger, H.L., 1983. Conditions for optimal growth of a PSTV-infected potato cell suspension and detection of viroid-complementary longer-than-unit-length RNA in these cells. *Plant Mol. Biol.* 2 (5), 239–247.
- Mühlbach, H.P., Sanger, H.L., 1979. Viroid replication is inhibited by alpha-amanitin. *Nature* 278 (5700), 185–188.
- Naoi, T., Kitabayashi, S., Kasai, A., Sugawara, K., Adkar-Purushothama, C.R., Senda, M., Hataya, T., Sano, T., 2020. Suppression of RNA-dependent RNA polymerase 6 in tomatoes allows potato spindle tuber viroid to invade basal part but not apical part including pluripotent stem cells of shoot apical meristem. *PLoS One* 15 (7), e0236481.
- Navarro, B., Flores, R., Di Serio, F., 2021. Advances in viroid-host interactions. *Annu. Rev. Virol.* 8 (1), 305–325.
- Navarro, B., Gisel, A., Rodio, M.E., Delgado, S., Flores, R., Di Serio, F., 2012. Viroids: how to infect a host and cause disease without encoding proteins. *Biochimie* 94 (7), 1474–1480.
- Navarro, J.A., Vera, A., Flores, R., 2000. A chloroplastic RNA polymerase resistant to tagetitoxin is involved in replication of avocado sunblotch viroid. *Virology* 268 (1), 218–225.
- Pei, Y., Shuman, S., 2002. Interactions between fission yeast mRNA capping enzymes and elongation factor Spt5. *J. Biol. Chem.* 277 (22), 19639–19648.
- Pieler, T., Rudt, F., 1997. Nucleocytoplasmic transport of 5S ribosomal RNA. *Semin. Cell Dev. Biol.* 8 (1), 79–82.
- Proudfoot, N.J., Brownlee, G.G., 1976. 3' non-coding region sequences in eukaryotic messenger RNA. *Nature* 263 (5574), 211–214.
- Qi, Y., Ding, B., 2003. Differential subnuclear localization of RNA strands of opposite polarity derived from an autonomously replicating viroid. *Plant Cell* 15 (11), 2566–2577.
- Qi, Y., Pellissier, T., Itaya, A., Hunt, E., Wassenegger, M., Ding, B., 2004. Direct role of a viroid RNA motif in mediating directional RNA trafficking across a specific cellular boundary. *Plant Cell* 16 (7), 1741–1752.
- Rackwitz, H.R., Rohde, W., Sanger, H.L., 1981. DNA-dependent RNA polymerase II of plant origin transcribes viroid RNA into full-length copies. *Nature* 291 (5813), 297–301.
- Rasmussen, E.B., Lis, J.T., 1993. *In vivo* transcriptional pausing and cap formation on three Drosophila heat shock genes. *Proc. Natl. Acad. Sci. USA* 90 (17), 7923–7927.
- Shi, Y., Manley, J.L., 2015. The end of the message: multiple protein-RNA interactions define the mRNA polyadenylation site. *Genes Dev.* 29 (9), 889–897.
- Spiesmacher, E., Mühlbach, H.P., Schnolzer, M., Haas, B., Sanger, H.L., 1983. Oligomeric forms of potato spindle tuber viroid (PSTV) and of its complementary RNA are present in nuclei isolated from viroid-infected potato cells. *Biosci. Rep.* 3 (8), 767–774.
- Strasser, K., Masuda, S., Mason, P., Pfannstiel, J., Oppizzi, M., Rodríguez-Navarro, S., Rondon, A.G., Aguilera, A., Struhl, K., Reed, R., Hurt, E., 2002. TREX is a conserved complex coupling transcription with messenger RNA export. *Nature* 417 (6886), 304–308.
- Suzuki, T., Ikeda, S., Kasai, A., Taneda, A., Fujibayashi, M., Sugawara, K., Okuta, M., Maeda, H., Sano, T., 2019. RNAi-mediated down-regulation of dicer-like 2 and 4 changes the response of 'moneymaker' tomato to potato spindle tuber viroid infection from tolerance to lethal systemic necrosis, accompanied by up-regulation of miR398, 398a-3p and production of excessive amount of reactive oxygen species. *Viruses* 11 (4), 344.
- Takeda, R., Petrov, A.I., Leontis, N.B., Ding, B., 2011. A three-dimensional RNA motif in potato spindle tuber viroid mediates trafficking from palisade mesophyll to spongy mesophyll in *Nicotiana benthamiana*. *Plant Cell* 23 (1), 258–272.
- Taylor, J.M., 2015. Hepatitis D virus replication. *Cold Spring Harb. Perspect. Med.* 5 (11), a021568.
- Thibaut, O., Claude, B., 2018. Innate immunity activation and RNAi interplay in citrus exocortis viroid-tomato pathosystem. *Viruses* 10 (11), 587.
- Tian, B., Graber, J.H., 2012. Signals for pre-mRNA cleavage and polyadenylation. *Wiley Interdiscip. Rev. RNA* 3 (3), 385–396.
- Verhoeven, J.T.J., Meekes, E.T.M., Roenhorst, J.W., Flores, R., Serra, P., 2013. Dahlia latent viroid: a recombinant new species of the family pospiviroidae posing intriguing questions about its origin and classification. *J. Gen. Virol.* 94 (Pt 4), 711–719.
- Wagner, S.D., Yakovchuk, P., Gilman, B., Ponicsan, S.L., Drullinger, L.F., Kugel, J.F., Goodrich, J.A., 2013. RNA polymerase II acts as an RNA-dependent RNA polymerase to extend and destabilize a non-coding RNA. *EMBO J.* 32 (6), 781–790.
- Wang, Y., 2021. Current view and perspectives in viroid replication. *Curr. Opin. Virol.* 47, 32–37.
- Wang, Y., Ding, B., 2010. Viroids: small probes for exploring the vast universe of RNA trafficking in plants. *J. Integr. Plant Biol.* 52 (1), 28–39.
- Wang, Y., Qu, J., Ji, S., Wallace, A.J., Wu, J., Li, Y., Gopalan, V., Ding, B., 2016. A land plant-specific transcription factor directly enhances transcription of a pathogenic noncoding RNA template by DNA-dependent RNA polymerase II. *Plant Cell* 28 (5), 1094–1107.
- Wang, Y., Zirbel, C.L., Leontis, N.B., Ding, B., 2018. RNA 3-dimensional structural motifs as a critical constraint of viroid RNA evolution. *PLoS Pathog.* 14 (2), e1006801.
- Wen, Y., Shatkin, A.J., 1999. Transcription elongation factor hSPT5 stimulates mRNA capping. *Genes Dev.* 13 (14), 1774–1779.
- Wu, J., Leontis, N.B., Zirbel, C.L., Bisaro, D.M., Ding, B., 2019. A three-dimensional RNA motif mediates directional trafficking of Potato spindle tuber viroid from epidermal to palisade mesophyll cells in *Nicotiana benthamiana*. *PLoS Pathog.* 15 (10), e1008147.
- Wu, X., Liu, M., Downie, B., Liang, C., Ji, G., Li, Q.Q., Hunt, A.G., 2011. Genome-wide landscape of polyadenylation in arabidopsis provides evidence for extensive alternative polyadenylation. *Proc. Natl. Acad. Sci. USA* 108 (30), 12533–12538.
- Zheng, Y., Wang, Y., Ding, B., Fei, Z., 2017. Comprehensive transcriptome analyses reveal that potato spindle tuber viroid triggers genome-wide changes in alternative splicing, inducible trans-acting activity of phasiRNAs and immune responses. *J. Virol.* 91 (11), e00247. -17.
- Zhong, X., Archual, A.J., Amin, A.A., Ding, B., 2008. A genomic map of viroid RNA motifs critical for replication and systemic trafficking. *Plant Cell* 20 (1), 35–47.
- Zhong, X., Tao, X., Stombaugh, J., Leontis, N., Ding, B., 2007. Tertiary structure and function of an RNA motif required for plant vascular entry to initiate systemic trafficking. *EMBO J.* 26, 3836–3846.