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Plant subviral RNAs as a long noncoding RNA (lncRNA): Analogy with animal lncRNAs in host–virus interactions

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ABSTRACT

Satellite RNAs (satRNAs) and viroids belong to the group called subviral agents and are the smallest pathogens of plants. In general, small satRNAs and viroids are 300–400 nt in size and do not encode any functional proteins; they are thus regarded as so-called long noncoding RNAs (lncRNAs). These lncRNAs are receiving great attention as a new RNA class involved in gene regulation to control important biological processes such as gene transcription and epigenetic regulation. A substantial number of lncRNAs in animal cells have been found to play important roles in the interactions between a virus and its host. We here discuss the pathogenicity of subviral RNAs (especially satRNAs) in plant cells and their functions as lncRNAs associated with viral diseases, using animal lncRNAs as an analogy. Because, unlike animal lncRNAs, plant subviral RNAs can replicate and accumulate at very high levels in infected cells, we here considered the unique possibility that the RNA silencing machinery of plants, an important defense mechanism against virus infection, may have brought about the replication ability of subviral molecules. In addition, we also discuss the possibility that satRNAs may have arisen from plant–virus interactions in virus-infected cells. Understanding the molecular functions of these unique lncRNAs in plants will enable us to reveal the most plausible origins of these subviral RNAs.

1. Introduction

According to the ICTV classification (King et al., 2012), subviral agents in plants, which are not "viruses" in a precise sense, are divided into viroids and satellites. Satellites comprise the satellite viruses and satellite nucleic acids. Satellite nucleic acids are further divided into satellite DNAs and satellite RNAs (satRNAs). The satRNAs are either double-stranded satRNAs or single-stranded satRNAs (ss-satRNAs).

Considering the pathogenicity and evolutionary history of subviral agents beyond this classification, viroids and small ss-satRNAs share the common feature of pathogenic, short, single-stranded RNAs without any functional open reading frames. From the point of view of functional and regulatory short RNAs, viroids and satRNAs certainly belong to the so-called long noncoding RNAs (lncRNAs), which are longer than 200 nt and thus quite different from the well-known small RNAs with 20–30 nt.

In animals, lncRNAs have been extensively studied because they play important roles in human cell differentiation and disease development through functions such as the regulation of gene transcription (as discussed in detail in other chapters). At least four mechanisms for transcriptional regulation of genes have been proposed for lncRNAs, including signaling for gene activation, acting as molecular decoys (mimicries) for gene suppression, guiding RNA–protein complexes to the correct chromatin positions and serving as scaffolds in complex formation (Wang and Chang, 2011).

Although functional studies of lncRNAs in plants are still in the early stages of research, as in animals, lncRNAs in plants seem to regulate gene expression and be involved in plant biological processes such as flowering, male sterility, nutrition metabolism and stress responses (Zhang et al., 2013; Zhang and Chen, 2013). The best-known example is polycomb repressive complex 2 (PRC2) associated with chromatin remodeling, which is recruited by lncRNAs to the correct chromatin positions (Tsai et al., 2010; Wang and Chang, 2011) and catalyzes histone methylation to suppress gene expression. In the regulation of flowering, lncRNA COLDAIR, which is induced by vernalization (cold treatment), recruits PRC2 to the *FLC* loci and suppresses the expression of *FLC*, resulting in flowering induction (Heo and Sung, 2011).

2. Viroids and satRNAs as lncRNAs

The ability to multiply and accumulate to high levels in infected plant cells is unique to viroids and small ss-satRNAs as plant lncRNAs. To our knowledge, no lncRNAs have been found to multiply in

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animal cells. If any lncRNA is ever found to multiply in animal cells, there will be no distinction between animal and plant lncRNAs.

Plant satRNAs are either linear or circular ssRNA molecules, which replicate in the cytosol using the replication system of the helper virus. In addition, satRNA depends on the helper virus for its encapsidation for transmission (Simon et al., 2004; Hu et al., 2009). Because satRNAs generally decrease the level of the helper virus, they mostly attenuate disease symptoms induced by their helpers and thus satRNA are regarded as "molecular parasites of viruses". Intriguingly, some satRNAs enhance the symptoms induced by their helpers even if the level of the helper virus is decreased. Viroids, on the other hand, are circular ssRNAs and autonomously replicate in either nuclei or chloroplasts using host polymerase (Flores et al., 2014). In both satRNAs and viroids, unlike known animal and plant lncRNAs, there is no sequence homology with other organisms including plant genomes or helper virus genomes; thus, their origins remain a matter of speculation and great interest for plant virologists. Viroids and satRNAs actually share some common replication features. For example, by using the agroinfiltration approach, helper virus-independent replication of *Cucumber mosaic virus* (CMV) satRNA in nucleus has been demonstrated (Choi et al., 2012; Seo et al., 2013; Chaturvedi et al., 2014). In addition, Rao and Kalantidis (2015) recently proposed that satRNA had two (viroids-like and virus-like) replication phases.

3. Classification of plant single-stranded satRNAs

Single-stranded satRNAs essentially comprise three major groups: (1) large, linear ss-satRNA; (2) small, linear ss-satRNA; (3) small, circular ss-satRNA. The first category, the large, linear ss-satRNAs have genomes that are 0.7–1.5 kb in size and encode a nonstructural protein. *Bamboo mosaic virus* (BaMV) satRNA is grouped in this category and has been most studied and revealed much important information on the nature of satRNAs (Wang et al., 2014). BaMV satRNAs actually encode an RNA-binding protein (P20), which is involved in viral movement (Palani et al., 2006). Whether a certain RNA sequence in BaMV satRNA plays a regulatory role is still unknown. The small, linear genome of the ss-satRNAs in the second category is less than 0.7 kb and does not encode functional proteins. The small, circular ss-satRNAs in the third category are found in infected cells in both circular and linear forms and have been given the general name "virusoids" because of their similarity to viroids. *Tobacco ringspot virus* satRNA has been studied in detail for its ribozyme activity (Chay et al., 1997; Nelson et al., 2005). Recently, *Rice yellow mottle virus* (RYMV) satRNA categorized in the third group was found to encode a possible peptide whose function is not yet determined (AbouHaidar et al., 2014). Because ribozymes have self-catalytic activity and are thus regarded as an lncRNA (Pyle, 2014), the third group satRNAs should be also regarded as an lncRNA in plants whether they can produce a peptide or not. There is a similar circular satRNA in human pathogens. *Hepatitis delta virus* (HDV), which is dependent on *Hepatitis B virus* for its replication and encapsidation, and thus actually an ss-satRNA, may be considered to be an lncRNA because it has a ribozyme necessary for its replication (Been and Wickham, 1997).

Here, we will mainly focus on the second category of satRNAs in our analogy with lncRNAs because more information on their molecular functions is available. Among the second category of satRNA, we focus on CMV satRNAs, the most studied of this group, and for which the molecular interactions among satRNA, helper virus (CMV) and host plant are best understood (Palukaitis and García-Arenal, 2003; Jacquemond, 2012; Kouadio et al., 2013). CMV satRNAs are 330–400 nt in size and do not encode any proteins. Most CMV satRNAs can attenuate CMV symptoms and are thus called benign satRNAs and sometimes used for CMV attenuation because CMV disease control is very important for many agricultural crops. On the other hand, some CMV satRNAs drastically change and even worsen CMV symptoms and are thus called malignant satRNAs. Such malignant CMV satRNAs can induce lethal systemic necrosis on tomato and chlorosis (yellowing) on tobacco or tomato and have been intensively studied at the molecular level. For example, the nucleotide sequences responsible for those symptom modifications have been studied in many laboratories (Masuta and Takanami, 1989; Kuwata et al., 1991; Jaegle et al., 1990; Devic et al., 1990; Sleat et al., 1994). The yellowing symptoms on tobacco induced by CMV Y-satRNA (Y-sat) have been implicated in RNA silencing-based gene regulation (Wang et al., 2004). In systemic necrosis on tomato, CMV D-satRNA has been reported to be involved in programmed cell death that requires a plant hormone, ethylene (Xu and Roossinck, 2000; Irian et al., 2007).

4. Gene expression in the symptom modification induced by CMV satRNAs

4.1. Yellowing symptoms induced by siRNA derived from CMV Y-sat

Following the finding that the Y-sat-mediated yellowing symptoms might be induced through host RNA silencing (Wang et al., 2004), two simultaneously published studies on CMV Y-sat (Shimura et al., 2011; Smith et al., 2011) finally demonstrated that short-interfering RNAs (siRNAs) derived from Y-sat could control gene expression in infected plant cells, inducing distinct symptom modulation. The two groups verified that the bright yellowing symptoms on tobacco plants infected with CMV+Y-sat were essentially induced by Y-sat-derived siRNAs, leading to down-regulation of chlorophyll biosynthetic gene *ChlI* (Fig. 1). A unique 22-nt sequence region (SYR) complementary to the *ChlI* mRNA was located in the center of the Y-sat sequence (369-nt long). Transgenic plants that express the silencing-resistant variant *ChlI* mRNA or plants infected with a mutant Y-sat in which the SYR region is modified no longer developed the yellowing symptoms, suggesting that this phenomenon is indeed sequence-specific. Because CMV satRNAs generally accumulate a large amount of siRNA in infected cells through dsRNAs as both replicative intermediate and secondary structures of ssRNA (Du et al., 2007), it is likely that an enormous amount of Y-sat siRNAs caused a spontaneous trigger of RNA silencing against a particular gene. Because the SYR region in Y-sat has not been found in the other CMV satRNAs, Y-sat is considered to have coincidentally acquired the SYR region in its evolutionary history. Because aphids are drawn to yellow color by nature, Y-sat-mediated symptom modulation (yellowing of tobacco leaves) may have been advantageous to Y-sat survival by attracting the insect vector.

Here, we can draw a high degree of analogy between Y-sat-mediated yellowing and a gene regulation system in animal cells in terms of mechanism of lncRNA function and disease induction. Briefly, microRNA-155 (miR-155), an oncomiR that can cause breast cancer by targeting the *socs1* gene, is generated from the lncRNA called BIC, which is greatly expressed after retrovirus infection (Jiang et al., 2010; Kung et al., 2013). In our comparison, Y-sat and SYR-derived siRNA correspond, respectively, to BIC and miR-155. Y-sat is abundantly amplified in CMV+Y-sat-infected cells, leading to the induction of yellowing, while BIC expression is enhanced by retrovirus infection to cause cancer of human cells.

4.2. Mechanism of CMV satRNAs to enhance host defense

Attenuation of CMV that harbors satRNA has been explained by competition for replication machinery between the helper virus (i.e., CMV) and satRNA (Simon et al., 2004). Although this