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FACULTY OF AGRICULTURAL SCIENCES & ALLIED INDUSTRIES

Satellite RNAs and Satellite Viruses of Plants

1. Introduction

Several viruses, as obligate parasites to the host plants, are associated with even smaller molecular parasites, sometimes being commensal or even beneficial, and are among the simplest life forms, namely, satellite RNAs (satRNAs) and satellite viruses. These satRNAs are short RNA molecules, usually <1,500 nt, that depend on cognate helper viruses for replication, encapsidation, movement, and transmission, but most share little or no sequence homology to the helper viruses. In contrast, satellite viruses are satRNAs that encode and are encapsidated in their own capsid proteins (CPs).

Certain satRNAs code for nonstructural proteins, but most satRNAs do not encode any functional protein products and are therefore thought to exert their biological functions through direct RNA interactions. Recent advances in research into satRNAs and satellite viruses have resulted in deeper insights into the molecular biology of these small replicating entities and to certain practical applications in modern biotechnology.

Satellite viruses and satRNAs have attracted much interest over the past decades, mainly for the following reasons:

(1) They can modulate − attenuate or exacerbate − the symptoms caused by their cognate helper viruses

(2) They do not encode their own RNA-dependent RNA polymerases (RdRps) for their own replication and apparently use replication machineries similar to those of the helper viruses and thus have great potential as surrogate systems for the study of the replication mechanisms of their cognate helper viruses;

(3) They can alter − usually reduce − the accumulation of their cognate helper viral RNAs and are thus considered the molecular parasites of the helper viruses; and

(4) They can accumulate to high levels in host plants and thus in some cases can be developed into high-level expression vectors for foreign genes.

Because of these features, satRNAs and satellite viruses are good biological systems for the study of

the molecular biology of viruses. Indeed, current studies of satRNAs and satellite viruses have provided further insights into several key subjects in molecular virology. In addition, recent studies have revealed the roles of an antiviral defense system of host plants, RNA silencing or posttranscriptional gene silencing (PTGS) in the pathogenicity and molecular biology of satRNAs. This information has altered our views of satRNAs and satellite viruses as being purely parasitic to the helper viruses.

2. General classification of satRNAs and satellite viruses

To assist in distinguishing the different categories of sub-viral RNAs, an illustrated key is provided in Figure 1. Each category of subviral RNA can be identified according to the salient features listed in the key by the detection of additional RNAs from the purified virus particles (virions) or from virions with different morphology and/or density. The satellites, which are categorized at a status equivalence of "family", can be classified into the following types:

Single-stranded RNA satellite viruses,
Single-stranded satellite DNAs,
Double-stranded satRNAs,
Single-stranded satRNAs.

Single-Stranded RNA Satellite Viruses

There are two subgroups in this type:

subgroup 1, Chronic bee-paralysis virus-associated satellite virus, which contains a single member, Chronic bee-paralysis satellite virus

subgroup 2, Tobacco necrosis satellite viruses, which include the four members Maize white-line mosaic satellite virus, Panicum mosaic satellite virus, Tobacco mosaic satellite virus and Tobacco necrosis satellite virus. With respect to conventions, in the following section, these satellite viruses are referred to by names or acronyms traditionally used among plant virologists; for example, Panicum mosaic satellite virus is referred to as Satellite panicum mosaic virus (SPMV) in recent publications. The four species of satellite viruses in subgroup 2 are associated with helper viruses in the genera

Aureusvirus, Panicovirus, Tobamovirus, and Necrovirus. These satellite viruses share no sequence similarities with each other, which suggests that they might have originated from independent events in evolutionary history. Among them, only Satellite tobacco mosaic virus (STMV) has a rod-shaped virus as the helper. Of note, deletion of nucleotides A and G at positions 1 and 61, respectively, of STMV, which is naturally adapted to *Tobacco mild green mosaic virus*, is required for adaptation to other helper viruses, namely *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* or *Green tomato atypical mosaic virus*. Satellite viruses can co-evolve with the helper viruses whenever they encounter each other.

Single-stranded satRNAs

Single-stranded satRNAs are classified into three subgroups:

Subgroup 1, large satRNAs;
subgroup 2, small linear satRNAs;
subgroup 3, circular satRNAs.

Subgroup 1 contains large satRNAs of about 0.7 to 1.5 kb that encode at least one nonstructural protein. Thus, satRNAs in this subgroup are usually referred to as messenger-type satRNAs. Species in subgroup 1 recognized by the International Committee on Taxonomy of Viruses include Arabis mosaic virus large satRNA (satArMV), Bamboo mosaic virus satRNA (satBaMV), Chicory yellow mottle virus large satRNA, Grapevine Bulgarian latent virus satRNA, Grapevine fanleaf virus satRNA (satGFLV), Myrobalan latent ringspot virus satRNA, Strawberry latent ringspot virus satRNA, Tomato black ring virus (TBRV) satRNA, TBRV G serotype satRNA, and Beet ringspot virus satRNA. Although the actual biological functions of many nonstructural proteins encoded by satRNAs are unknown, the protein encoded by TBRV satRNA has been detected *in vivo* and is involved in its replication. The P3 protein encoded by a large satRNA, designated RNA3, of 1,114 nt, and associated with GFLV-F13, is also involved in satRNA replication. In addition, the protein encoded by the satArMV is required for the *in planta* replication of the satRNA. In contrast, the P20 protein encoded by satBaMV RNA is not essential for replication but is involved in the systemic movement of satBaMV RNA. It preferentially binds to 5' and 3' untranslated regions (UTRs) of satBaMV RNA and interacts with the CP, a movement protein of its helper virus [triple gene block protein 1 (TGBp1)] and P20 itself. The subcellular localization and expression kinetics of P20 have recently been investigated in

detail, which further suggests its involvement in long-distance movement of satBaMV RNA. In addition to the satRNA-encoded proteins with reported functions, *Blackcurrant reversion virus* in the genus *Nepovirus* of the family *Comoviridae* also contains a satRNA, which is 1,432 nt and encodes a single protein of 402 amino acids and has unknown function.

Species in subgroup 2 of single-stranded satRNAs include Cucumber mosaic virus (CMV) satRNA, Cymbidium ringspot virus (CymRSV) satRNA, Pea enation mosaic virus satRNA, GRV satRNA, Panicum mosaic virus small satRNA, Peanut stunt virus (PSV) satRNA, Turnip crinkle virus (TCV) satRNA, and Tomato bushy stunt virus (TBSV) satRNA B10, and TBSV B1 [15,33]. Tobacco necrosis virus small satRNA and Robinia mosaic virus satRNA are listed as tentative species in this subgroup. The satRNAs in this subgroup are short (usually less than 700 nt), linear RNA molecules that do not exhibit any biologically significant messenger activity. Among the short linear satRNAs, those associated with TCV, CMV, and PSV are the most thoroughly characterized. Strictly speaking, one of the satRNAs associated with TCV, namely satC, does not fit the definition of satRNAs, because satC is a hybrid molecule composed of a true satRNA, satD, and two fragments from the 3' terminus of TCV genomic RNA. However, studies of the satC have revealed many important factors and mechanisms involved in the infection cycle of both TCV and satC, which will be discussed in detail in the following sections.

Subgroup 3 includes the following species: ArMV small satRNA, Cereal yellow dwarf virus-RPV satRNA (previously Barley yellow dwarf virus satellite RNA), Chicory yellow mottle virus satRNA, Lucerne transient streak virus satRNA, Solanum nodiflorum mottle virus satRNA, Subterranean clover mottle virus satRNA (2 types), Tobacco ringspot virus satRNA, and Velvet tobacco mottle virus satRNA. Species in this subgroup are characterized by a small (shorter than 400 nt), circular RNA genome without biologically significant messenger activity. These circular satRNAs, previously referred to as virusoids, replicate through a rolling-circle mechanism and self-cleave into monomers by use of endogenous hammerhead ribozyme activity.

Figure. Schematic representation of the complex interaction among host plants, helper viruses, satRNAs and satellite viruses, and the RNA silencing mechanism of the host. T-shaped lines indicate inhibitory effect, whereas the solid arrows represent an enhancing effect. Arrows on dotted lines represent an "uncertainty state," in which the three participating members (host plants, helper viruses, and satRNAs or satellite viruses) would

have to compete for the targeting of host gene silencing mechanisms. **B** Example scenarios illustrating the consequences of competition among plants, viruses, and satellites for the activation and targeting of the RNA silencing mechanisms. The yellow box indicates the emergence of new satRNAs as a result of an RNA silencing mechanism.

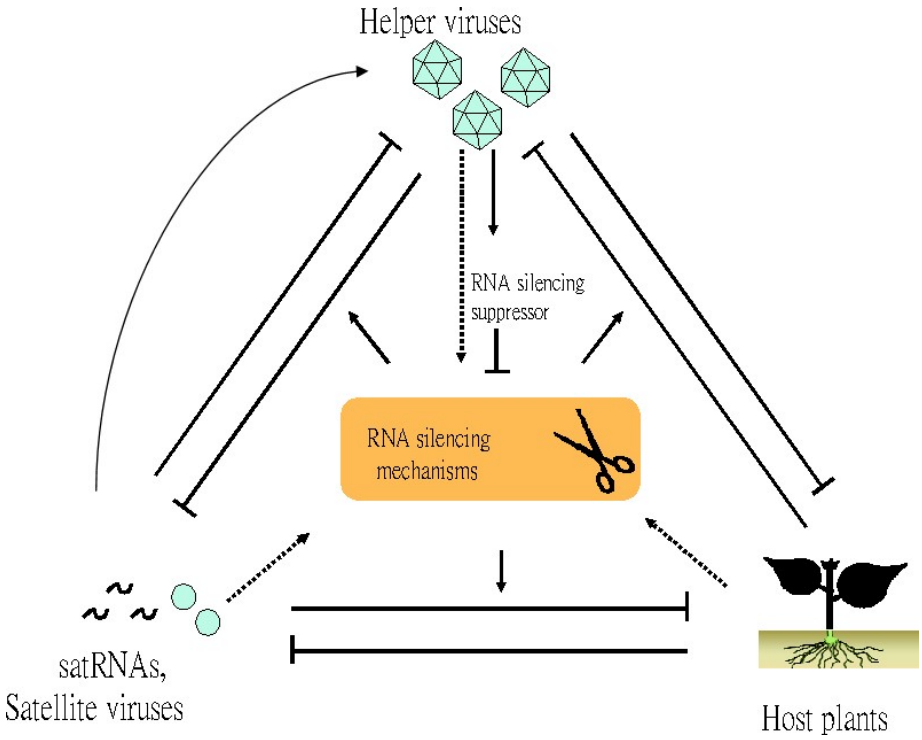
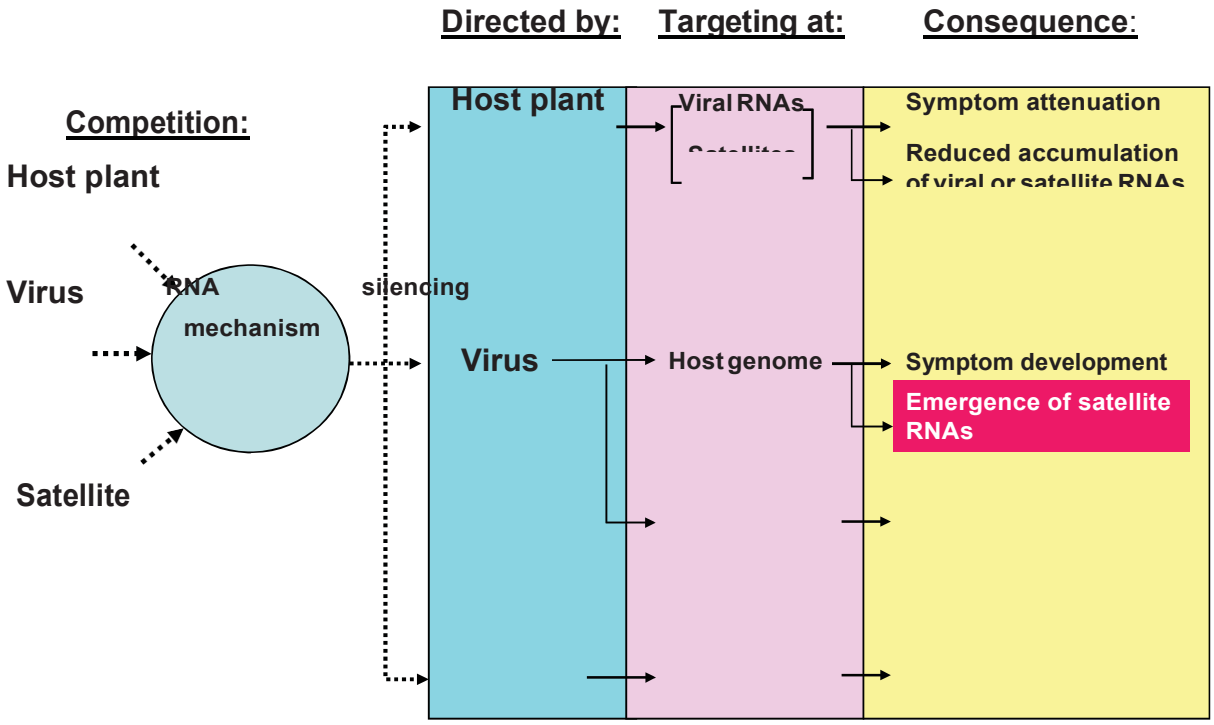


Figure 2. *Cont.*



Practical applications of satRNAs

Apart from the contribution of satRNAs in basic research into molecular virology, satRNAs have also been used in application-oriented studies. One of their potential applications lies in the development of satRNA-based vector systems for the expression of foreign genes in plants. In comparison with using other types of vectors for foreign gene expression, such as plasmids and viral vectors, using satRNAs and satellite viruses as vectors has the following advantages: i) ease of manipulation, ii) high *in vivo* stability, and iii) high expression level. First, because of the relatively small size of satRNAs and satellite viruses, they are simpler systems to use for cloning, sequencing, genetic modification, and regular maintenance. Second, most satRNAs are highly structured and thus significantly more resistant to degradation by nucleases *in vivo* than are other viral RNA-based vector systems. For instance, about 49% of the genome of one of the most thoroughly characterized satCMVs participates in the formation of base-paired structures, which increases the robustness of the satRNA *in planta*. And finally, most satRNAs and satellite viruses can accumulate to high levels in host plants, which leads to the increase in level of proteins translated from the messenger-type RNAs of the satellites. However, despite the aforementioned potentials, most attempts to develop vectors based on coding or non-coding satRNAs for applications in biotechnology have failed, probably because of the strict requirement for the maintenance of certain secondary structures, which severely limited the sequence alterations that could be introduced. The current applications of satRNAs are demonstrated only for the following rare cases:

1) As vectors for *in planta* expression of foreign genes:

The practical application of satBaMV RNA was demonstrated more than ten years ago [38], and remains the sole example of such applications. Being a messenger-type satRNA, satBaMV RNA encodes a P20 gene, which, unlike all other large messenger-type satRNAs, was shown to be nonessential for the replication of satRNA. Therefore, P20 was replaced with a chloramphenicol acetyltransferase (CAT) gene, which resulted in high expression of the CAT protein in infected *Chenopodium quinoa* and demonstrated that satBaMV RNA could be useful as a satellite-based expression vector.

2) As vectors for functional studies:

satBaMV RNA has been used for the expression of individual TGBps in a complementary experiment for study of cell-to-cell movement of BaMV. To dissect the functions of individual TGBps, single or multiple TGBps of BaMV, PVX and *Foxtail mosaic virus* were expressed by use of satBaMV RNA-based vectors to complement the functions of green fluorescent protein-tagged, movement-defective BaMV with mutation(s) in the matching gene(s). The results revealed the requirement for species-specific interactions among TGBps for cell-to-cell movement of BaMV and possibly other potexviruses. In addition, a satBaMV-based vector system has been used in analysis of promoter sequences to generate potexvirus subgenomic RNAs (sgRNAs). Insertion of subgenomic promoter-like sequences (SGPs) into the upstream of the start codon of the P20 gene gave rise to the synthesis of sgRNA of satBaMV in infected cells co-inoculated with helper BaMV RNA. By deletion and mutational analyses, one core promoter-like sequence, two upstream enhancers and one downstream enhancer were identified in the function of SGP *in vivo*. Such applications in functional analysis further demonstrated the additional advantages of satRNA-based vectors: i) Applicable for mutational studies of essential genes or regulatory sequences: satRNAs are physically separated from the viral genomes and are not essential for the infection cycle of the helper viruses; thus, they can be used in mutational or complementary assays of regulatory sequences. ii) Convenient for combinational studies on multiple genes: two or more satRNA-based vectors harboring different genes may co-exist in one plant and be supported by the same helper virus, although they might be not expressed at the same level.

3) As vectors for gene silencing:

Gossele and Metzlaff described the use of STMV vectors for gene silencing. The work was an extension of their previous work with a satellite virus-induced silencing system (SVISS). The authors demonstrated the potential of STMV as a vector of gene silencing by knocking out (or knocking down) the expression in *N. tabacum* of a variety of genes involved in different biochemical pathways (e.g., phytoene desaturase, glutamine synthetase, chalcone synthase, and transketolase). Most of the silenced phenotypes could be observed 10-12 days

postinoculation, which indicates the efficiency of STMV-based vectors in the induction of gene silencing.

In addition, the symptom-attenuation features of satRNAs have been successfully exploited in developing satRNA-based disease-management systems, either directly, with satRNAs used as biological control agents, or indirectly, by producing transgenic plants that express satRNA sequences. The occurrence of some virus strains that do not support the replication of satRNAs in certain host plants may present an obstacle to satRNA-mediated disease management. However, satRNAs still provide us with experimental tools for understanding the complex trilateral interaction among satRNAs, helper viruses, and host plants.