Post-transcriptional gene silencing in plants

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Summary

Post-transcriptional gene silencing (PTGS) in plants is an RNA-degradation mechanism that shows similarities to RNA interference (RNAi) in animals. Indeed, both involve double-stranded RNA (dsRNA), spread within the organism from a localised initiating area, correlate with the accumulation of small interfering RNA (siRNA) and require putative RNA-dependent RNA polymerases, RNA helicases and proteins of unknown functions containing PAZ and Piwi domains. However, some differences are evident. First, PTGS in plants requires at least two genes – *SGS3* (which encodes a protein of unknown function containing a coil-coiled domain) and *MET1* (which encodes a DNA-methyltransferase) – that are absent in *C. elegans* and thus are not required for RNAi. Second, all *Arabidopsis* mutants that exhibit impaired PTGS are hypersusceptible to infection by the cucumovirus CMV, indicating that PTGS participates in a mechanism for plant resistance to viruses. Interestingly, many viruses have developed strategies to counteract PTGS and successfully infect plants – for example, by potentiating endogenous suppressors of PTGS. Whether viruses can counteract RNAi in animals and whether endogenous suppressors of RNAi exist in animals is still unknown.

Key words: Post-transcriptional gene silencing, RNA interference, Transgene, Virus, Mutants

Introduction

Epigenetic regulation of gene expression is a heritable change in gene expression that cannot be explained by changes in gene sequence. It can result in the repression or activation of gene expression and is therefore referred to as gene silencing or gene activation respectively. Until the end of the 1980s, only modifications of DNA or protein that lead to transcriptional repression or activation, or to the formation of prions, were classified as epigenetic (Lewin, 1998). During the 1990s, however, a number of gene-silencing phenomena that occur at the post-transcriptional level were discovered in plants, fungi, animals and ciliates, introducing the concept of post-transcriptional gene silencing (PTGS) or RNA silencing (Baulcombe, 2000; Matzke et al., 2001). PTGS results in the specific degradation of a population of homologous RNAs. It was first observed after introduction of an extracopy of an endogenous gene (or of the corresponding cDNA under the control of an exogenous promoter) into plants (Napoli et al., 1990; Smith et al., 1990; van der Krol et al., 1990). Because RNAs encoded by both transgenes and homologous endogenous gene(s) were degraded, the phenomenon was originally called co-suppression. A similar phenomenon in the fungus *Neurospora crassa* was named quelling (Romano and Macino, 1992; Cogoni et al., 1996). Later, several groups showed that PTGS can also affect transgenes that are not homologous to endogenous genes, suggesting that this phenomenon is not a simple regulatory mechanism that controls the expression of endogenous genes (Dehio and Schell, 1994; Ingelbrecht, 1994; Elmayan and Vaucheret, 1996). Fire et al. recently identified a related mechanism, RNA interference (RNAi), in animals (Fire et al., 1998). RNAi results in the specific degradation of endogenous RNA in the presence of homologous dsRNA either locally injected or transcribed from an inverted-repeat transgene (Tavernarakis et al., 2000). Injected dsRNA, as well as transgenes expressing dsRNA, also triggers silencing of homologous (trans)genes in plants (Chuang and Meyerowitz, 2000; Schweizer et al., 2000; Waterhouse et al., 1998). This strongly suggests that a mechanistic link between PTGS, quelling and RNAi exists. Here, we focus on the molecular characteristics of PTGS, its dynamics, its genetic dissection and its role in resistance to viruses in plants.

PTGS results in RNA degradation after transcription

PTGS greatly reduces mRNA accumulation in plant cytoplasm but does not affect transcription (de Carvalho et al., 1992; van Blockland et al., 1994). Detailed analyses of RNA content in plants exhibiting PTGS has revealed the presence of discrete RNA degradation intermediates. For example, in glucanase (trans)genes, both longer and smaller RNAs were found. The longer RNAs result from aberrant processing, whereas the smaller RNAs correspond to subfragments of the mRNA which suggests that degradation starts with an endonucleolytic cleavage followed by exonuclease digestion (van Eldik et al., 1998). In the case of chalcone synthase (trans)genes, Metzlaff et al. proposed that internal complementary regions form a dsRNA structure by pairing, which leads to the production of RNA degradation intermediates after endonucleolytic cleavage at both ends of such RNA duplexes (Metzlaff et al., 1997). Significant accumulation of sense and antisense siRNAs (approximately 20-25 nucleotides long) was observed in various PTGS systems in plants (Hamilton and Baulcombe, 1999). The accumulation of both sense and antisense siRNAs suggests that dsRNA is produced prior to RNA degradation. How dsRNAs are produced is still not completely understood, but the finding that a gene encoding an RNA-dependent RNA polymerase (RdRP) is required for PTGS (Mourrain et al., 2000; Dalmay et al., 2000) suggests that this enzyme is

involved in this process (see below). Studies of RNAi in *Drosophila* revealed that siRNAs result from the cleavage of the injected dsRNA and serve as guide to target the degradation of homologous mRNA (Zamore et al., 2000; Bernstein et al., 2001; Elbashir et al., 2001).

Initiation, propagation and maintenance of PTGS

The study of how PTGS is triggered has revealed the existence of at least three steps: initiation, propagation and maintenance. Indeed, spontaneous triggering of PTGS of nitrate reductase, nitrite reductase or SAM-synthase (trans)genes (which leads to particular chlorotic or necrotic phenotypes that correlate with the disappearance of the corresponding RNA) starts with interveinal or vein-localized spots on one leaf and then propagates to the upper leaves, in which it is subsequently maintained (Boerjan et al., 1994; Palauqui et al., 1996). These non-clonal patterns were found reproducibly in all transgenic lines in which a given gene was silenced, suggesting that the dissection of spontaneous PTGS into localized initiation, systemic propagation and active maintenance is a general rule.

Initiation

Because spontaneous initiation of PTGS in transgenic plants is localized and stochastic, it is particularly difficult to study. Most data concerning the control of initiation are indirect and result from the analysis of parameters that increase or decrease the efficiency of spontaneous triggering of PTGS. Such studies have revealed that two types of transgene loci efficiently trigger PTGS. The first type corresponds to highly transcribed single transgene copies. Several arguments suggest that the efficiency of triggering could depend on the probability that the transgene produces a particular form of RNA above a threshold level. Indeed, PTGS is triggered mostly when plants are homozygous for the transgene locus (de Carvalho et al., 1992). In addition, PTGS is triggered more efficiently when strong promoters are used (Que et al., 1997). Finally, PTGS is inhibited when transgene transcription is blocked (Vaucheret et al., 1997). The second type of transgene loci that efficiently triggers PTGS is those carrying two transgene copies arranged as an inverted repeat (IR). These IRs are usually transcribed at very low levels, which argues against the threshold model (van Blockland et al., 1994). To explain their ability to efficiently trigger PTGS, investigators have proposed that these IRs produce dsRNA by read-through transcription and that dsRNA efficiently triggers PTGS, even when produced at a low level. Indeed, introduction of single transgene copies that have a panhandle structure (i.e. carry the same sequence cloned in sense and antisense orientations downstream of the promoter) leads to efficient silencing of homologous (trans)genes, which suggests that such dsRNAs are efficient initiators of PTGS (Hamilton et al., 1998; Waterhouse et al., 1998).

The above results coincided with the discovery of RNAi in animals, a process that results in specific RNA degradation induced by injection of homologous dsRNA (Fire et al., 1998) or expression of panhandle transgenes (Tavernarakis et al., 2000). These similarities suggest that PTGS in plants and RNAi in animals could derive from an ancestral mechanism allowing degradation of RNAs that are homologous to dsRNAs abnormally present in a cell. However, the PTGS mechanisms triggered by highly transcribed single transgene loci and transgene IRs in plants are (at least in part) different. Indeed, mutants in which PTGS triggered by highly transcribed single transgene copies is impaired exhibit efficient PTGS triggered by transgene IRs (H.V. and P. Waterhouse, unpublished). This suggests that highly transcribed single transgene loci do not directly produce dsRNA and that the mutants that have been isolated are impaired in the steps leading to the formation of dsRNA (see below).

Systemic propagation

The transmission of PTGS of nitrate reductase, nitrite reductase or SAM-synthase (trans)genes from localized interveinal spots or vein-localized to the upper leaves of plants suggested that a PTGS propagation signal exists. The existence of such a signal was clearly established by grafting (Fig. 1). Silencing was transmitted with 100% efficiency from silenced stocks to target scions expressing the corresponding transgene but not to scions expressing a non-homologous transgene, which indicates that the signal is sequence-specific (Palauqui et al., 1997). Silencing of nitrate-reductase genes was also transmitted to a non-transgenic mutant scion overexpressing the endogenous *Nia2* gene owing to metabolic derepression but not to a wild-type scion, which indicates that overaccumulation of Nia mRNA above the level of that in wild-type plants, rather than the presence of a transgene in the scion, is required for triggering of RNA degradation during PTGS (Palauqui and Vaucheret, 1998). The transmission of PTGS also occurred when silenced stocks and non-silenced target scions were physically separated by up to 30 cm of stem of a non-target wild-type plant, indicating long-distance propagation (Palauqui et al., 1997). Voinnet and co-workers drew similar conclusions when PTGS of a GFP transgene was systemically triggered after they inoculated one leaf of a non-silenced GFP transgenic *N. benthamiana* plant with an *Agrobacterium* strain carrying the GFP transgene (Voinnet and Baulcombe, 1997) or biolistically introduced the GFP transgene (Voinnet et al., 1998). Because it is sequence specific and mobile, this signal could be made (at least in part) of RNA. Whether it corresponds to dsRNA or siRNA remains to be determined.

Maintenance

Grafting experiments using nitrate-reductase-silenced tobacco stocks and a set of different transgenic and non-transgenic scions revealed similar requirements for spontaneous initiation and maintenance. Indeed, when grafting-induced silenced scions were removed from the silenced stocks and regrafted onto wild-type plants, silencing was not maintained in lines that cannot trigger PTGS spontaneously (Fig. 1, class I and III plants). These lines seem to be able to ‘sense’ the systemic PTGS signal that induces the degradation of the mRNA, but cannot (re)produce the signal. Conversely, silencing was maintained in transgenic lines that are able to trigger PTGS spontaneously (Fig. 1, class II plants), which indicates that only the transgene loci that are able to initiate PTGS can maintain a silent state (Palauqui and Vaucheret, 1998). The ability of a transgenic line to (re)produce the systemic silencing signal could depend on the genomic location and/or the structure of the transgene locus, which would thus involve a nuclear step in PTGS. Chemical modifications (e.g. DNA methylation or histone acetylation) or structural modification (i.e. chromatin remodeling) could correspond to an epigenetic imprint induced by the systemic silencing signal,

allowing PTGS to be actively maintained during development. This imprint could be maintained in newly developing tissues in a conservative manner during replication or could be imposed de novo in each new cell in response to the systemic signal. Recently, our group showed that a mutant in which the major maintenance DNA-methyltranferase was impaired exhibited impaired maintenance of PTGS (Morel et al., 2000), which supports this hypothesis (see below).

The experiments described above clearly show that PTGS is a dynamic process that can be separated into initiation, propagation and maintenance. However, a number of points remain mysterious. In particular, the nature of the systemic silencing signal remains to be determined.

Genetic dissection of PTGS

PTGS mutants in plants and other organisms

To identify PTGS mutants, several groups have mutagenized *Arabidopsis* lines carrying silent transgenes. Dehio and Schell identified mutants in which the efficiency of silencing of a 35S-rolB transgene is increased and named these enhancers of gene silencing (*egs*), defining two genetic loci: *egs1* and *egs2* (Dehio and Schell, 1994). Subsequently, we isolated mutants in which silencing of a 35S-GUS transgene was inhibited, named suppressor of gene silencing (*sgs*), which define at least three genetic loci (Elmayan et al., 1998; Mourrain et al., 2000). During the same screen, we also identified PTGS mutants impaired in the previously identified *AGO1* gene (Fagard et al., 2000). Furthermore, Dalmay et al. have isolated mutants in which silencing of a 35S-GFP transgene by a PVX-35S-GFP amplicon is inhibited; these are named silencing-defective (*sde*) and define four genetic loci (Dalmay et al., 2000). Simultaneously, *Neurospora* mutants exhibiting impaired quelling were isolated and named quelling-defective (*qde*; Cogoni and Macino, 1997), and *Caenorhabditis elegans* mutants exhibiting impaired RNAi were isolated and named RNAi-defective (*rde*; Tabara et al., 1999) or mutator (*mut*; Ketting et al., 1999). The cloning of the corresponding genes revealed that PTGS, quelling and RNAi share common steps, and provided further insights into these processes at the molecular level.

Genes that inhibit PTGS in plants

Despite their identification in 1994, the *EGS1* and *EGS2* genes (which are presumed to encode proteins that negatively regulate PTGS) have not been cloned yet. Nevertheless, a tobacco gene that negatively regulates PTGS has been recently identified. This gene, named *rgs-CaM* (for regulator of gene silencing), encodes a calmodulin-related protein (Anandalakshmi et al., 2000), and its overexpression in tobacco inhibits PTGS of a 35S-GUS transgene (see below). However, its role in wild-type plants is still not known.

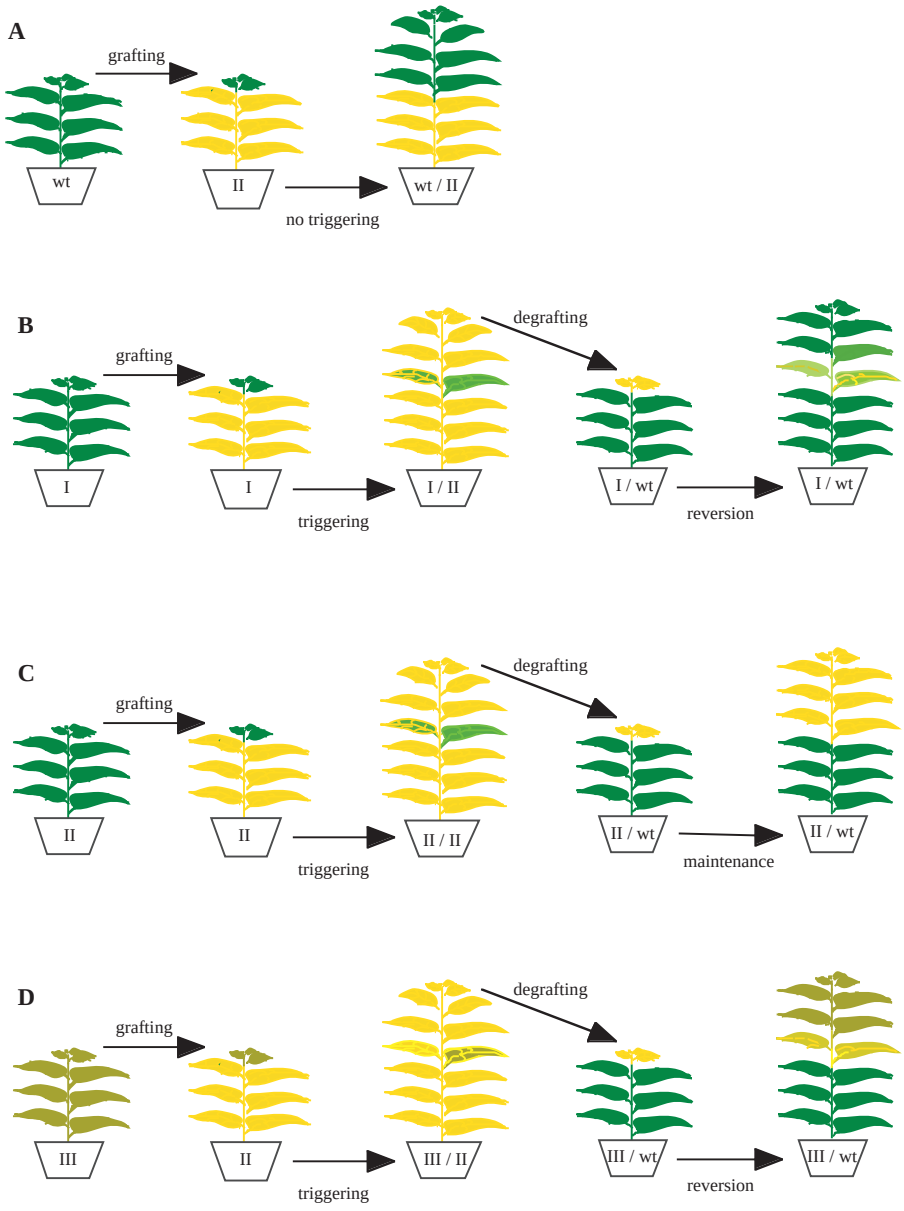

Fig. 1. Evidence for a systemic silencing signal and for a maintenance step in PTGS. (A) Wild-type plants (wt) do not undergo PTGS after grafting onto silenced transgenic plants. (B) Transgenic plants that do not spontaneously undergo PTGS (class I) undergo PTGS after grafting onto silenced transgenic plants but do not maintain silencing after elimination of the silenced rootstock. (C) Non-silenced transgenic plants derived from lines that can spontaneously undergo PTGS (class II) undergo PTGS after grafting onto silenced transgenic plants and maintain silencing after elimination of the silenced rootstock. (D) Non-transgenic plants that express an endogenous gene at high level owing to metabolic derepression (class III) undergo PTGS after grafting onto silenced transgenic plants but do not maintain silencing after elimination of the silenced rootstock.

Genes that stimulate PTGS in plants

Our group has shown that the *Arabidopsis sgs* mutants are deficient for both PTGS of an exogenous 35S-GUS transgene and PTGS of a homologous 35S-*Nia2* transgene and endogenous *Nia* genes, but not PTGS induced by transgenes producing dsRNA (Elmayan et al., 1998; Mourrain et al., 2000; H.V. and P. Waterhouse, unpublished). Dalmay et al. showed that the *Arabidopsis sde1* and *sde3* mutants are deficient for PTGS of a 35S-GFP transgene induced by a PVX-35S-GFP amplicon but not for virus-induced gene silencing (VIGS; see below; Dalmay et al., 2000; Dalmay et al., 2001). This indicates that *SGS* and *SDE* genes positively control PTGS induced by highly transcribed transgenes but not PTGS induced by IR transgenes or viruses. Deficiency of PTGS in *sgs* and *sde* mutants correlates with a strong decrease in methylation of the transcribed region of the transgene, which confirms the correlation between PTGS and methylation. The *sgs* mutations have no effect on transgenes silenced at the transcriptional level, which indicates that they are specific for PTGS (Elmayan et al., 1998; Mourrain et al., 2000).

The *Arabidopsis SGS2* gene (and the *SDE1* gene, which is identical to *SGS2*) encodes a protein that has strong similarity to a tomato RdRP (Mourrain et al., 2000; Dalmay et al., 2000). The existence of an RdRP activity in plants was known for 30 years but its role was not. SGS2/SDE1 is similar to QDE-1, which is required for quelling in *Neurospora* (Cogoni and Macino, 1999), and EGO-1, which is required for RNAi of some genes in the germline of *C. elegans* (Smardon et al., 2000). The *Arabidopsis SGS3* gene encodes a protein that has no significant similarity to other known proteins in plants or other kingdoms (Mourrain et al., 2000). Its function cannot be deduced, because it does not contain any known protein motif other than a coiled-coil domain present in the C-terminus of the protein, which suggests possible interactions with other proteins. The absence of similar proteins in *C. elegans* and *Drosophila* (two organisms that exhibit RNAi and whose genomes are entirely sequenced) and the absence of the corresponding mutant in *Neurospora* suggest that the function of the SGS3 protein is specific to plant PTGS.

A third gene that positively controls PTGS in *Arabidopsis* corresponds to a previously identified gene controlling development, *AGO1* (Fagard et al., 2000). *ago1* mutants display strong developmental alterations that affect plant architecture and fertility. The AGO1 protein shares similarity with a number of proteins containing Piwi and PAZ (Piwi/Argonaute/Zwille) domains (Cerutti et al., 2000): QDE-2, required for quelling in *Neurospora* (Catalanotto et al., 2000); RDE-1, required for RNAi in *C. elegans* (Tabara et al., 1999); eIF2C, presumed to play a role in the control of translation initiation in rabbit (Zou et al., 1998); STING, required for silencing of the repetitive *Stellate* locus in *Drosophila* (Schmidt et al., 1999); and PIWI, required for germline maintenance in *Drosophila* (Cox et al., 1998). Recently, the *SDE3* gene that positively controls PTGS in *Arabidopsis* was isolated. It encodes an RNA helicase that shares similarity with MUT-6, which is required for PTGS in *Chlamydomonas* (Wu-Scharf et al., 2000), and SMG-2, which is required for RNAi in *C. elegans* (Domeier et al., 2000). Therefore, despite the absence of orthologs of *SGS3*, the identification of different sets of related proteins (SGS2/QDE-1/EGO-1, AGO1/QDE-2/RDE-1, SDE3/SMG-2) indicates that PTGS, quelling and RNAi probably derive from the same ancestral mechanism.

The influence of chromatin and methylation genes on plant PTGS

The *Arabidopsis* mutants *ddm1* and *met1* were isolated from a screen for mutations that result in a general reduction (~70%) in methylation of the genome (Vongs et al., 1993). *MET1* encodes the major DNA methyltransferase (Finnegan et al., 1996). *DDM1* encodes a protein related to SNF2/SWI2 chromatin-remodelling proteins (Jeddeloh et al., 1999), which suggests that structural changes in chromatin can reduce the accessibility of DNA to the methylation machinery. Both *ddm1* and *met1* mutants exhibit impaired TGS (Steimer et al., 2000; Morel et al., 2000). Furthermore, they also exhibit impaired PTGS, which correlates with a decrease in transgene methylation (Morel et al., 2000). However, unlike *sgs* and *ago1* mutants, *ddm1* and *met1* mutants do not show impaired PTGS in all plants. In addition, the impairments of PTGS in *ddm1* and *met1* mutants are different: in *ddm1* mutants PTGS is inhibited in the whole plant throughout its life, whereas in *met1* mutants, PTGS is progressively inhibited during the course of plant development. This suggests that *MET1* and *DDM1* are involved in the maintenance and initiation steps of PTGS, respectively. Together, these results confirm the existence of a nuclear step in PTGS and reveal a genetic link between PTGS and TGS.

A branched model for PTGS in plants

Several cellular components involved in the control of PTGS in plants have been identified. By extrapolation of genetic and biochemical results obtained in *Neurospora*, *C. elegans* and *Drosophila* to plants, we propose a branched model for PTGS in plants (see Fig. 2):

1. PTGS induced by highly transcribed single transgene loci could be initiated by transcription of aberrant RNAs (abRNAs). Although such abRNAs have not been isolated, their existence is supported by the effect of the *ddm1* mutation in the triggering of PTGS. Indeed, the absence of the DDM1 protein at an early step of development could induce changes in chromatin conformation that impede the production of abRNAs by the transgene.

2. The RdRP protein encoded by the *SGS2/SDE1* gene could use such abRNAs as templates to synthesize dsRNA. SGS2 could also play a role in the production of the systemic silencing signal and/or its amplification.

3. The SGS3, AGO1 and SDE3 proteins, like SGS2/SDE1, are not required for PTGS induced by IR transgenes or viruses. They could facilitate the RdRP activity of SGS2/SDE1, by impeding translation of abRNAs, allowing them to be used as templates to synthesize dsRNA.

4. Unidentified plant RNases, similar to DICER, which is involved in RNAi in *Drosophila* (Bernstein et al., 2001), could participate in the degradation of dsRNA and in the formation of siRNAs. *Arabidopsis* mutants impaired in a gene sharing strong similarities with the *Drosophila DICER* gene – *caf* (also known as *sin1* or *sus1*; Jacobsen et al., 1999; A. Ray, personnal communication) are currently being analyzed to determine whether it plays a role similar to that of DICER in plants. siRNAs could subsequently direct the RNA-degradation complex (named RISC in *Drosophila*) to homologous mRNAs, allowing completion of their degradation.

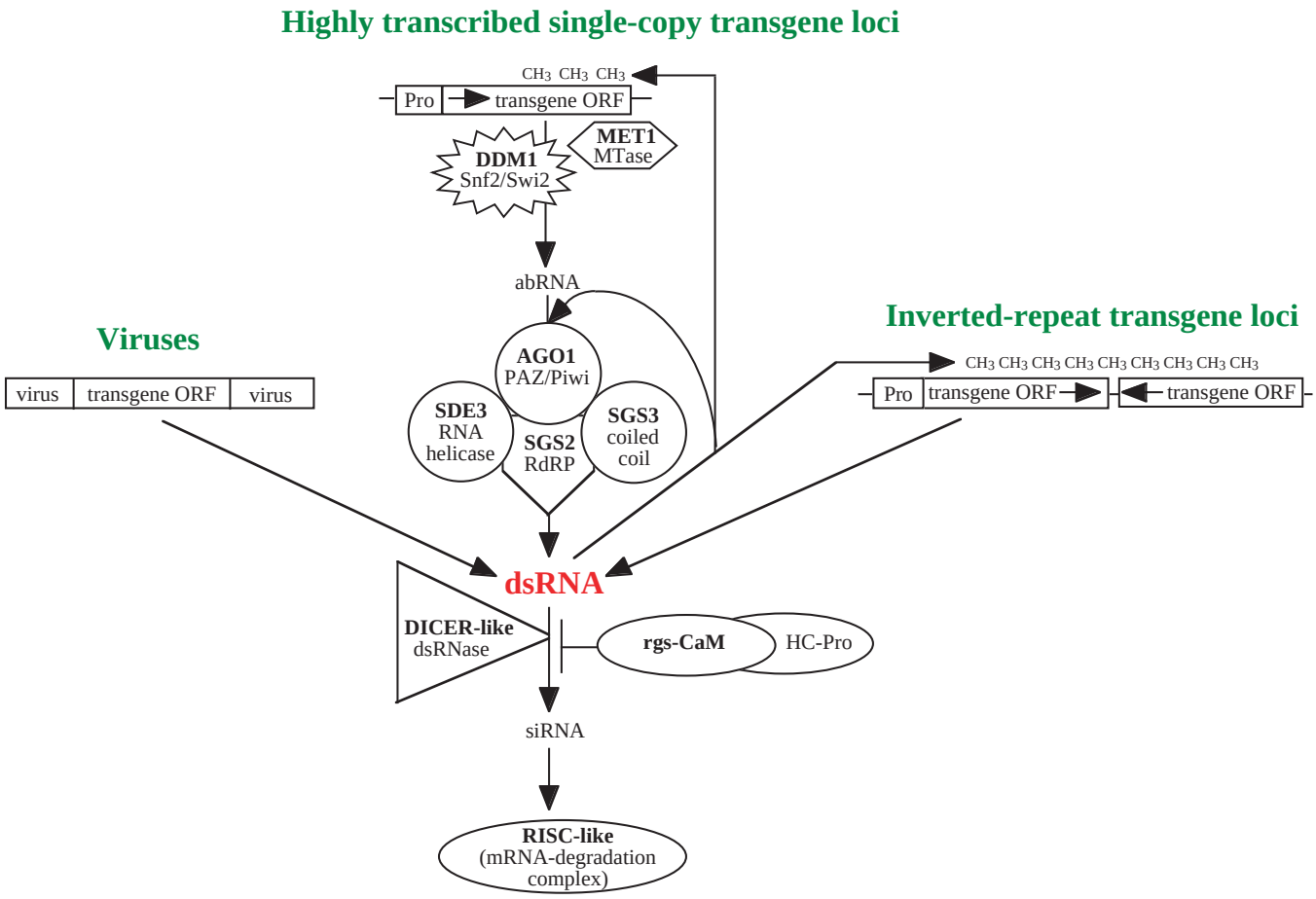

Fig. 2. A model for PTGS in plants. Transgene loci arranged as inverted repeats or viruses can directly produce dsRNA. Like dsRNA injected in animals, they can be cut by dsRNase (e.g. the DICER enzyme of *Drosophila*), thus generating siRNAs. The latter could target mRNA to an RNA-degradation complex (named RISC in *Drosophila*). Transgene loci carrying a single copy expressed at high level could transcribe abRNAs owing to changes in chromatin structure induced by DDM1. These abRNAs could be used as templates by an RdRP (SGS2/SDE1) to synthesize dsRNAs. This RdRP could be helped by two proteins of unknown function (SGS3 and AGO1) and an RNA helicase (SDE3), which are not required for PTGS induced by IR transgene loci that directly produce dsRNA. The dsRNAs could also induce methylation of transgene DNA (involving MET1), thus reinforcing its ability to produce abRNAs.

5. The MET1 protein could be necessary for the maintenance of PTGS during plant development, methylating transgene sequences that are homologous to dsRNAs, and thus maintaining the chromatin state that is responsible for the synthesis of abRNA. Indeed, Wassenegger et al. have shown that dsRNAs direct DNA methylation of homologous sequences in the nucleus (Wassenegger et al., 1994).

PTGS and plant resistance to viruses

Since the discovery of PTGS, a number of experiments have revealed that there is a complex relationship between PTGS and virus infection/resistance. Indeed, viruses can be targets, inducers or inhibitors of PTGS.

Viruses as targets of PTGS

Introduction of transgenes constitutively expressing part of the genome of a virus can lead to resistance of the plant to infection by this virus (reviewed by Marathe et al., 2000). Plants can either resist infection (resistance is then referred to as immunity) or undergo a preliminary phase of infection from which they recover (resistance is then referred to as recovery; Lindbo et al., 1993; Smith et al., 1994). Plants in which the transgene undergoes PTGS prior to infection are immune, whereas plants in which the transgene undergoes PTGS after infection show recovery. This suggests that homologous virus and transgene RNA are degraded by a PTGS-like mechanism. Both resistant plants and plants that exhibit recovery are immune to secondary infection by the same virus or by another recombinant virus carrying part of the genome of the first virus, which indicates that plants have a ‘memory’ of the first virus. It is therefore tempting to hypothesize that this memory is based on the presence of a silencing signal similar to that revealed by grafting experiments.

Interestingly, recovery does not occur only in transgenic plants expressing part of the genome of a virus. In some cases, wild-type plants can recover from virus infection by specifically degrading virus RNA (Al-Kaff et al., 1998; Covey et al., 1997; Ratcliff et al., 1997; Ratcliff et al., 1999). Similarly to ‘recovered’ transgenic plants, these wild-type recovered plants are immune to secondary infection by the same virus or by another recombinant virus carrying part of the genome of

Fig. 3. PTGS-deficient mutants are hypersusceptible to infection by CMV. Non-infected PTGS-deficient *sgs* mutants of *Arabidopsis* (C) grow as non-infected wild-type plants (A), whereas disease symptoms are much more pronounced in CMV-infected mutants (D) compared with CMV-infected wild-type plants (B). Infected mutants have very small stems, are completely sterile and eventually die, whereas infected wild-type plants develop stems with elongated internodes and are still able to form seeds. The difference in symptom severity caused by CMV infection is due to an average fivefold overaccumulation of viral RNA in the mutants.

Fig. 4. Inhibition of PTGS by viruses. Introduction of a 35S-NiR transgene into tobacco can trigger PTGS of endogenous NiR genes, which leads to growth inhibition and leaf chlorosis (A). Infection by viruses such as CMV or TEV (a potyvirus) inhibits PTGS and restores the growth of plants (B).

the first virus, showing that the memory signal can be maintained despite the absence of homologous transgenes (Ratcliff et al., 1997; Ratcliff et al., 1999). These results suggested that PTGS participates in a mechanism for plant resistance to viruses, a hypothesis that was confirmed by the discovery that *sgs2/sde1, sgs3, sde3 and ago1* mutants are hypersusceptible to infection by a cucumovirus, CMV (Fig. 3; Mourrain et al., 2000; Dalmay et al., 2001; J.-B. Morel and H.V., unpublished). Therefore, there might be similarities between particular virus RNAs and transgene RNAs that make them targets for the PTGS machinery.

Viruses as inhibitors of PTGS

PTGS-deficient *sgs2/sde1, sgs3, sde3* and *ago1* mutants are hypersusceptible to infection by CMV, which leads to overaccumulation of CMV RNA. However, RNA from potyvirus, tobamovirus or tobravirus accumulates at the same level in wild-type plants and *sgs2/sde1, sgs3* and *sde3* mutants, which indicates that viruses have probably developed efficient strategies to counteract or to escape PTGS (Dalmay et al., 2000; Mourrain et al., 2000). Indeed, many viruses can inhibit PTGS (Anandalakshmi et al., 1998; Béclin et al., 1998; Brigneti et al., 1998; Kasschau and Carrington, 1998; Voinnet et al., 1999; Fig. 4). In particular, the potyvirus TuMV and the tobamovirus TVCV totally inhibit PTGS in *Arabidopsis*, whereas CMV only partially inhibits PTGS (Mourrain et al., 2000). The proteins responsible for PTGS inhibition by several viruses have been identified. These proteins are able alone to inhibit PTGS, even in the absence of the virus. Interestingly, no similarities between these different inhibiting proteins have been identified. How these proteins inhibit PTGS remains mostly unknown. In the case of the HC-Pro protein of the potyviruses, PTGS inhibition seems to result from activation of the cellular *rgs-CaM* gene (Anandalakshmi et al., 2000). Indeed, the accumulation of *rgs-CaM* mRNA is induced by virus infection or in plants expressing HC-Pro. In addition, expression of *rgs-CaM* under the control of a strong promoter is sufficient to inhibit PTGS in the absence of virus. Expression of HC-Pro does not inhibit methylation or the production of a systemic silencing signal but prevents the accumulation of the siRNAs, which suggests that it acts downstream of both the signal and the induction of methylation and upstream of the production of siRNAs (Mallory et al., 2001). In the case of the p25 protein of potexviruses, Voinnet et al. have proposed that

the propagation of the systemic silencing signal of PTGS is inhibited (Voinnet et al., 2000).

Viruses as inducers of PTGS

As mentioned above, recovery can be induced after virus infection, indicating that viruses can trigger PTGS. The induction of PTGS by viruses was confirmed by the observation that endogenous genes or transgenes can be silenced after infection with recombinant viruses carrying part of the (trans)gene sequence. This phenomenon is called virus-induced gene silencing (VIGS; Kjemtrup et al., 1998; Kumagai et al., 1995; Ruiz et al., 1998; Ratcliff et al., 2001). In the case of VIGS directed against transgenes, plants recover from virus infection, but transgene silencing and methylation persist in the absence of the virus (Ruiz et al., 1998; Jones et al., 1999). This suggests that VIGS induces the production and the propagation of a silencing signal in the uninfected parts of the plant, which triggers transgene silencing and immunity against the virus. In contrast, in the case of VIGS directed against endogenous genes, the virus persists in plants, and the endogenous gene remains unmethylated (Ruiz et al., 1998; Jones et al., 1999). This suggests that endogenous genes cannot (re)produce the silencing signal. These results are reminiscent of the previous results obtained in grafting experiments in which grafting-induced silencing is not maintained in a mutant overexpressing the endogenous *Nia2* gene in the absence of the silencing rootstock (Fig. 1). Considering the involvement of methylation in the maintenance of PTGS (Morel et al., 2000), one might imagine that endogenous genes cannot maintain and produce the silencing signal, because they are not susceptible to methylation by homologous RNA.

The identification of a plant defence strategy based on the degradation of virus RNA is an important discovery of the 1990s. However, plant-virus interactions that are regulated at the RNA level are complex, because viruses can be targets, inducers or inhibitors of PTGS. Considering that PTGS and VIGS can be inhibited by viruses, we must now determine precisely how PTGS can degrade virus RNA and how viruses can induce VIGS. These apparent contradictions could be explained by the dynamics of infection. In particular, the differential spread speed and location of both the virus and the systemic silencing signal (Voinnet et al., 2000) could determine whether the virus or the plant wins the battle.

Concluding remarks

The mechanisms and biological roles of epigenetic regulation are now emerging. Until recently, TGS and PTGS were considered to be separate pathways controlling different targets. However, this dogma has been recently broken. First, it was shown that dsRNAs act as key regulators that trigger either TGS or PTGS, depending on whether they are homologous to promoter or coding sequences (Waterhouse et al., 1998; Mette et al., 2000). Second, components that regulate both TGS and PTGS (*DDM1* and *MET1*) were identified in plants (Morel et al., 2000). Interestingly, silent transposons are reactivated in *Arabidopsis ddm1* mutants that exhibit impaired TGS and PTGS (Hirochika et al., 2000; Singer et al., 2001; Miura et al., 2001) and in *C. elegans* and *Chlamydomonas* mutants that exhibit impaired PTGS or RNAi (Ketting et al., 1999; Wu-Scharf et al., 2000). These observations therefore suggest that silencing phenomena derive from an ancestral mechanism and that they have evolved in different kingdoms to assume different biological roles. Since they are active against transposons, viruses and/or transgenes, their main function seems to be related to the control of invading nucleic acids. To date, there are no data indicating that viruses are influenced by PTGS in animals, which suggests that viruses elicit different defence mechanisms in plants and animals. Indeed, DNA viruses mostly integrate into the animal genome and could be controlled mainly by TGS. Alternatively, this could reflect the absence of data. The fact that PTGS was originally discovered in plants eleven years ago, and that RNAi was discovered in animals three years ago, is an excellent example of the importance that should be given to the comparative study of epigenetics in different kingdoms.

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