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The Silencing of (Trans)Genes – A mechanism of Virus Resistance in Plants

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Gene silencing and RNA-mediated virus resistance are two remarkable and potentially useful phenomena that occur in higher plants. However, in light of recent research it seems likely that the post-transcriptional type of gene silencing and RNA-mediated virus resistance are actually manifestations of the same phenomenon. The occurrence of both post-transcriptional gene silencing (PTGS) and RNA-mediated virus resistance require sequence homology between a transgene and an endogenous gene or a transgene and an infecting virus, respectively. Furthermore, both processes are characterized by high transcription rates of homologous, silenced (trans)genes but low steady-state levels of their transcripts (in case of virus-infected plants, low steady-state transcript levels of the silenced transgene and homologous viral RNA which eventually leads to virus resistance). Therefore, PTGS is a potential tool for creating virus-resistant transgenic plants that express a sequence homologous to the invading virus. It is very unlikely, though, that PTGS in plants has evolved solely for the purpose of transgene suppression so it is perhaps not surprising that some natural virus defense systems have been found to resemble gene silencing. In addition, although plants may combat virus infections by gene silencing, there is recent evidence that some plant viruses can fight back by suppressing the plant's ability to carry out the silencing process. The advantages and disadvantages of the commercial use of PTGS for creating virus-resistant plants (RNA-mediated virus resistance) is also discussed.

Key words: post-transcriptional gene silencing, RNA-mediated virus resistance, recovery, suppression of silencing.

It is now routine to construct transgenes with defined promoters and terminators and express them in plants, animals, fungi, etc. The stability of transgene expression is an important aspect in the development of e.g. transgenic plants for commercial applications. However, some introduced transgenes are not expressed as they should be. Thus, the phenomenon of gene silencing (for reviews see Matzke and Matzke, 1995, Baulcombe and English, 1996, Grant, 1999, Kooter et al., 1999) came as an unwelcome surprise to some. Gene silencing affects homologous sequences, for example, the presence of a transgene with sequence homology to an endogenous gene can sometimes lead to inactivation of one or both of these genes. This gene inactivation is a great disadvantage in applied plant biotechnology: expression of transgenes in higher plants can be inactivated by gene silencing by frequencies of up to 30% of independent transformants (Grant, 1999). Furthermore, a survey held in 1994 among thirty biotechnology companies revealed that almost all of them had encountered problems associated with gene silencing (Finnegan and McElroy, 1994).

Two types of gene silencing phenomena have been observed in plants, transcriptional gene silencing (no transcription of the silenced gene) and post-transcriptional gene silencing (transcription of the silenced gene but increased degradation of its transcript). The purpose of this review is to discuss:

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– similarities between the remarkable and potentially useful phenomena of gene silencing and RNA-mediated virus resistance that occur in higher plants,

– the use of gene silencing in producing virus-resistant crops.

Since RNA-mediated virus resistance is associated with post-transcriptional gene silencing (PTGS) (Lindbo et al., 1993, Mueller et al., 1995, van den Boogaart et al., 1998), transcriptional gene silencing will not be dealt here.

Some hallmarks of post-transcriptional gene silencing (PTGS)

One of the first interesting observations of PTGS in plants was reported in 1990, when two different research groups were trying to overexpress the chalcone synthase *(chs)* gene in petunia (Napoli et al., 1990, van der Krol et al., 1990). The *chs* gene encodes a key enzyme in flavonoid biosynthesis, the process responsible for both the production of antimicrobial compounds and giving flowers their colors. Both groups found that the flowers of a small percentage of the transformed plants were white instead of the expected deep purple color. Although the *chs* mRNA level in white flowers was significantly reduced, the rate of transcription of the transgene and endogenous *chs* gene (measured by analyses of RNA synthesized by isolated nuclei, i.e., in "run on" experiments) was not affected. These results suggested the occurrence of PTGS targeted against the *chs* gene and operating through the specific degradation of *chs* mRNA (van Blokland et al., 1994).

Another hallmark of PTGS is that the presence of a nuclear (trans)gene is not even a requirement for its occurrence. This was first demonstrated by Kumagai et al. (1995), who placed the gene encoding phytoene desaturase, an enzyme involved in the carotenoid biosynthesis pathway, under the control of a tobacco mosaic virus (TMV) subgenomic promoter in an infectious clone of TMV. Upon virus inoculation, these plants initially developed symptoms typical of a TMV infection. However, one to two weeks after inoculation, endogenous phytoene desaturase mRNA could not be detected. The suppression of phytoene desaturase expression was evident as visible photobleaching, due to the lack of carotenoid protection in photosynthesizing tissues. These experiments demonstrated that not only nuclear genes but cytoplasmic, replicating (e.g. viral) nucleic acids containing homologous sequences can also suppress endogenous gene expression.

The phenotype of a silenced gene usually is maintained through vegetative propagation or organ regeneration (Bruening, 1998, Palauqui and Balzergue, 1999). However, transmission of PTGS to progeny through meiosis is unpredictable, with silencing appearing among the progeny of a silenced plant with frequencies of 2–100%. Similarly, some progeny of a nonsilenced transgenic plant may be silenced (Dehio and Schell, 1994, Palauqui and Vaucheret, 1998, Wassenegger and Pelissier, 1998). Therfore, PTGS appears to be a meiotically unstable trait.

PTGS has been shown to be developmentally regulated (Dehio and Schell, 1994). When the *rolB* gene of *Agrobacterium rhizogenes* was expressed in *Arabidopsis thaliana,* young seedlings expressed the gene at a high level but later in the development

of the plant *rolB* expression was suppressed in a post-transcriptional manner. The induction of silencing was shown to depend on environmental conditions but once established it was maintained during subsequent development of the plant. The developmental regulation of PTGS was also demonstrated by Elmayan and Vaucheret (1996), who described *in planta* silencing of an integrated bacterial *UidA* gene (encoding ß-glucuronidase, GUS) cloned downstream of a double enhanced 35S promoter. The suppression was induced developmentally and showed a difference in the stage at which silencing was induced between homozygous and hemizygous plants. The homozygous plants showed the silenced state after four months of growth, whereas the hemizygous plants did not show any silencing for up to a year. In fact, this and other similar observations that transgene loci are silenced more strongly when homozygous than when hemizygous are extremely interesting from the perspective of plant breeding, because they suggest a role of gene silencing in the establishment of inbreeding depression and heterosis (Depicker and Van Montagu, 1997).

A remarkable property of PTGS in plants is that it is able to systemically spread (systemic acquired silencing) (Palauqui et al., 1997, Voinnet and Baulcombe, 1997). Grafting experiments have shown that silencing can be transmitted from silenced stocks to nonsilenced scions containing a transcriptionally active homologous (trans)gene (Palauqui et al., 1997). These experiments suggested that PTGS involves a transgene specific, diffusible messenger. Voinnet and Baulcombe (1997) came to the same conclusion with plants that express a gene from a jellyfish species encoding green fluorescent protein (GFP). When these plants were locally infected with *Agrobacterium tumefaciens* carrying the GFP transgene, silencing of GFP expression was induced first in the infected leaves but later it appeared also in upper uninfected leaves. Both groups suggested that a nucleic acid derived from the transgene might be the signal involved. The timing of PTGS spread indicates that the signal is sent out from the site of silencing induction before any transcript degradation can be detected. The above-mentioned two research groups monitored the silencing of a nitrate reductase gene *(Nia2)* and GFP, respectively, in transgenic plants and followed the kinetics of PTGS spread by removing leves in which silencing was induced by transient transformation (particle bombardment) with DNA homologous to the transgenes (Voinnet et al., 1998, Palauqui and Balzergue, 1999). If leaves were left on for two days or more, PTGS could spread systemically. This was several days before silenced phenotypes could be seen at the site of silencing induction.

Taken together, some of the more important hallmarks of PTGS in plants are:

– High transcription levels of the silenced gene but relatively low steady-state cytoplasmic levels of its transcript.

– Silencing occurs through a homology dependent mechanism: it suppresses identical or closely related sequences and is likely due to the activation of a sequence-specific RNA-degradation apparatus (discussed later).

– Silencing of a nuclear-derived sequence (transgene or endogene) can be triggered by either nuclear genes or by cytoplasmic replicating nucleic acids that contain homologous sequences.

– Silencing can be developmentally regulated and is meiotically unstable.

– A silenced state in a plant can be systemically propagated through transport of a diffusible signal (systemic acquired silencing).

RNA-mediated virus resistance

In the mid-1980s, the concept of pathogen-derived resistance of plants emerged (Sanford and Johnston, 1985). This concept predicts that expressing genetic material of a pathogen in a host will disrupt essential pathogenic processes of infecting microbes and result in resistance to the pathogen. The feasibility of this approach was first demonstrated with viruses by expressing various forms of coat protein genes, viral replicase genes and other sequences (for reviews see Baulcombe, 1996, Palukaitis and Zaitlin, 1997).

Initially it was thought that pathogen derived resistance operates only through expression of the transgene-derived protein, and such a mechanism was suggested by several reports (Powel-Abel et al., 1986, Lapidot et al., 1993, Brederode et al., 1995). However, it was subsequently showed that untranslatable viral transgenes are also able to confer resistance (de Haan et al., 1992, Lindbo and Dougherty, 1992, van der Vlugt et al., 1992). This type of resistance, based on the presence of RNA, rather than protein, became known as RNA-mediated resistance. It is characterized by a high level of resistance not being overcome by virus inoculum concentrations that are orders of magnitude greater than the concentrations that routinely infect wild type plants (Bruening, 1998). However, this resistance proved to be highly virus specific, effective only against viruses closely related to the virus that was the source of the transgene sequence (Baulcombe, 1996, Prins and Goldbach, 1996). In fact, RNA-mediated resistance is effective only against viruses with a high sequence homology (at least 88%) to the transgene (Mueller et al., 1995). The requirement of such a high sequence specificity (in other words, a narrow resistance spectrum) is certainly a practical disadvantage of this type of resistance.

One of the first reports on RNA-mediated virus resistance described transgenic tobacco plants that express the coat protein gene of tobacco etch virus (TEV) in an untranslatable form (Lindbo and Dougherty, 1992). Some of the transgenic plant lines showed extreme resistance but others displayed the so-called recovery phenotype. In such plants, a systemic infection initially occurred. However, each new leaf that subsequently appeared showed milder symptoms and lower virus concentrations. Eventually, symptomless and virus-free leaves emerged that were completely resistant to superinfection. TEV resistance turned out to be highly sequence specific, effective only against closely related virus strains. In inoculated leaves, steady-state mRNA levels of the TEV coat protein transgene had dropped about 5– to 8-fold, compared to uninoculated tissue at the same developmental stage (Dougherty et al., 1994). On the other hand, nuclear run-on experiments to assess transcription levels revealed an unaltered rate of transcription, suggesting a post-transcriptional suppression of the transgene mRNA. These results implied that a sequence-specific RNA degradation mechanism was triggered in the infected plants that affects both the mRNA of the transgene and the replicating viral RNA. The differences between the two phenotypes, recovery and resistance, correlated with the number of

integrated transgene copies (Goodwin et al., 1996). One to two copies resulted in recovery while three or more copies resulted in resistance.

The induction of RNA-mediated resistance has been shown to depend not only on the number of transgene copies but also on transgene length. When plants were transformed with small, untranslatable sequences (110 to 235 nucleotides long) of the nucleoprotein gene of tomato spotted wilt virus (TSWV), no resistance was observed (Pang et al., 1997). However, when the same sequences were fused to the nontarget GFP gene, resistance was observed. These results suggest that a critical length of the transgene is necessary to induce RNA-mediated resistance.

Developmental regulation of RNA-mediated resistance has also been described. Homozygous transgenic plants that express the nucleoprotein gene of TSWV were silenced earlier in development than hemizygous plants (Pang et al., 1996). Resistance induction was also dependent on environmental conditions.

Other typical features of RNA-mediated virus resistance include the observation that its appearance is unpredictable after passing through meiosis (Goodwin et al., 1996). Also, the resistance is maintained in protoplasts isolated from resistant plants (Lindbo and Dougherty, 1992, Sijen et al., 1996), which implies that RNA-mediated resistance is functional at the single cell level.

In summary, some of the more important characteristics of RNA-mediated virus resistance in plants are:

– it is a high level of virus resistance that is not easily overcome by a high inoculum dose.

– it is effective only against closely related viruses.

– RNA-mediated resistance requires a high sequence homology between the infecting virus and a viral-derived transgene in the host. It correlates with high levels of transgene transcription but low steady-state cytoplasmic levels of the transgene mRNA.

– the mechanism of resistance involves the activation of a sequence-specific RNA degradation apparatus (discussed later) that affects both the replicating viral RNA and the mRNA of the transgene.

– RNA-mediated resistance can be developmentally regulated and appears to be meiotically unstable.

The mechanisms behind RNA-mediated resistance are not entirely understood at present. However, it is apparent that this phenomenon of virus resistance shows similarities to post-transcriptional gene silencing (PTGS), the process that affects the expression of (trans)genes and homologous sequences.

Similarities between post-transcriptional gene silencing and RNA-mediated virus resistance

Post-transcriptional gene silencing (PTGS) and RNA-mediated virus resistance seem to have a lot of features in common. These include observations that the occurrence of both phenomena require sequence homology between a transgene and an endogenous

gene or a transgene and an infecting virus, respectively. Furthermore, in both cases post-transcriptionally inactivated (trans)genes are capable of inactivating homologous sequences.

A direct connection between RNA-mediated virus resistance and PTGS was demonstrated by the elegant experiments of English et al. (1996). The RNA genome of potato virus X (PVX) is tolerant to the insertion of an additional gene, although the burden of inserted nucleotide sequences slows the replication process (Chapman et al., 1992). PVX with an inserted GUS gene was infectious in wild type tobacco plants but not in transgenic plants that are post-transcriptionally silenced for GUS. However, PVX lacking the GUS sequence was infectious and did replicate in both wild type and transgenic (GUS) plants. Thus, a post-transcriptional process not only removed the genomic GUS mRNA but also prevented the replication of virus containing the same (GUS) sequence. Furthermore, tomato plants with a silenced polygalacturonase gene and tobacco plants carrying a silenced neomycin phosphotransferase gene likewise prevented replication of PVX when it carried a homologous sequence but allowed replication of the virus carrying other sequences. This seminal work (English et al., 1996) clearly demonstrated that the mechanism of PTGS can prevent virus replication in plants.

Another strong evidence for a link betweeen PTGS and RNA-mediated virus resistance comes from the work of Mueller et al. (1995) and English et al. (1996). Two types of transgenic lines were available, both containing the PVX gene coding for an RNA polymerase. The first type showed a highly strain-specific resistance against PVX and a low level of RNA polymerase mRNA, and had properties associated with post-transcriptional inactivation of the RNA polymerase transgene. The second type was susceptible to PVX-infection and displayed a higher level of RNA polymerase mRNA. When the progeny of a cross between two opposite types of transgenic lines was analyzed it was shown that the resistant line is capable of inactivating the homologous transgene in the susceptible line (Mueller et al., 1995). In other words, the resistant *and* silenced phenotype was dominant to the susceptible *and* nonsilenced phenotype.

Possible mechanisms of post-transcriptional gene silencing and RNA-mediated virus resistance

Two currently accepted models, not mutually exclusive, attempt to explain post-transcriptional gene silencing (PTGS) and RNA-mediated virus resistance in plants. The first involves a threshold level of RNA (Baulcombe, 1996, Prins and Goldbach, 1996, Vaucheret et al., 1998). This so-called *threshold, or quantitative model* proposes that the plant is somehow able to sense the mRNA level of the (trans)gene and, if the level is too high, the plant targets the mRNA for degradation. The hypothesis came from work on transgenic tobacco plants that contain an untranslatable TEV coat protein gene (Lindbo et al., 1993). As mentioned previously, two phenotypes were observed, resistance and recovery, that were related to the number of integrated copies of the transgene (Goodwin et al., 1996). Resistance was observed when three or more copies were present, while one

to two copies resulted in a recovery phenotype. The authors suggested that induction of the resistance phenotype was dependent on the transcription rate and the resulting mRNA level of the transgene. Three or more copies (high transcription rate) would lift the transgene mRNA level above the critical threshold to induce resistance. One or two copies (lower transcription rate), initiallly would not be sufficient to induce resistance. However, the RNA of the replicating virus combined with the transgene mRNA would be sufficient to reach the threshold and induce an RNA degradation mechanism. The authors further suggest that once the threshold level was reached, an RNA-dependent RNA polymerase (RdRP), present in the cytoplasm of plants, would produce short antisense RNA molecules with sequence homology to the target (transgene and virus) RNA. Annealing of these antisense RNA molecules would lead to the formation of a double-stranded RNA structure that could be a target for degradation by double-stranded RNA specific RNases.

The involvement of RdRP and double-stranded RNA in the degradation process of RNA described above is plausible in the light of some recent findings. RdRP-s have been known for some time to be present in plants (Fraenkel-Conrat, 1986), and an RdRP activated upon virus infection has recently been cloned from tomato (Schiebel et al., 1998). In addition, double-stranded RNA and transgenes that produce transcripts that can become double-stranded can induce gene silencing in plants (Hamilton et al., 1998, Waterhouse et al., 1998).

Further support for the quantitative, threshold level model of PTGS comes from other reports that describe the relationship between silencing and virus resistance (Smith et al., 1994, Goodwin et al., 1996, for a recent review see Waterhouse et al., 1999). In addition, differences between homozygous and hemizygous plants in developmental timing of PTGS can also be explained by the threshold level model (Elmayan and Vaucheret, 1996). The homozygous plants would have a higher transcription rate and mRNA level of the transgene, and thus induce the silenced state earlier in development compared with hemizygous plants.

On the other hand, there are a number of results that cannot be explained by the threshold level model. As mentioned previously, this model is based on the observation that silenced (trans)genes often have a much higher transcription rate than nonsilenced genes. This is not always the case, however. Van Blokland et al. (1994) found that silenced and nonsilenced *chs* genes were transcribed at similar rates. A similar result was reported with RNA-mediated virus resistance (Mueller et al., 1995, English et al., 1996), which led these authors to suggest another model for the mechanism of PTGS, the so-called *aberrant mRNA, or qualitative model.* This model involves a qualitative feature of mRNA also known as aberrancy (Baulcombe and English, 1996). Aberrant mRNA derived either from a transgene or a virus would be distinct from normal mRNA. This distinction in turn would somehow be recognized, which would lead to degradation of both the aberrant and homologous "normal" mRNA during PTGS.

What features would make an mRNA aberrant? As described earlier, English et al. (1996) found that replication of a recombinant PVX strain carrying the GUS gene is suppressed in plants which are transgenic and post-transcriptionally silenced for GUS. It

was shown that the target for virus resistance was in the 3' region of the GUS gene, which correlated with the methylation pattern of the transgene. The authors suggested that transgene methylation could be directly responsible for the production of a truncated *(aberrant)* mRNA because of transcript termination in the 3' methylated region. The production of an aberrant mRNA could also be caused by other features of the transgene (e.g. untranslatable, no introns, or other unusual characteristics). The structural aberrations could make aberrant mRNAs preferred templates for a plant-encoded RdRP. Thus, short antisense RNAs would be produced that target homologous sequences (including "normal" transgene mRNA and homologous viral RNA) for degradation through the formation of double-stranded RNAs, in a similar manner as described for the quantitative (threshold) model of PTGS.

The quantitative and qualitative models of PTGS certainly have some features in common. For example the idea of a qualitatively aberrant mRNA does not exclude the possibility that a quantitative element may simultaneously affect PTGS. Obviously, if more aberrant mRNA were produced, the RNA degradation mechanism would be more efficient. In fact, both models assume the presence of an RNA degradation mechanism (by involvement of a plant-encoded RdRP and double-stranded RNA). Support for such a mechanism comes from two different lines of research: it has been shown in silenced petunias containing a *chsA* transgene (Metzlaff et al., 1997) and in tobaccos containing a TEV coat protein gene (Tanzer et al., 1997) that silencing correlates with the presence of low molecular weight RNA fragments derived from degradation of *chsA* mRNA and TEV coat protein mRNA, respectively. However, it should be noted that although RNA degradation seems to be a pivotal feature of both PTGS and RNA-mediated virus resistance, there is still a lot to be uncovered about the underlying mechanism that govern these processes (for a recent discussion see Waterhouse et al., 1999).

Virus resistance by gene silencing – a naturally occurring phenomenon in plants

It is unlikely that PTGS in plants has evolved solely for the purpose of transgene suppression so it is perhaps not surprising that some natural virus defense systems have been found to resemble gene silencing. In other words, it seems that viruses can trigger silencing in the apparent absence of homologous nuclear (transgene) sequences. For example, infection of *Nicotiana clevelandii* by tomato blackring nepovirus (TBRV) strain W22 results in an initial symptomatic phase in which the virus moves systemically, followed by a recovery state in which new tissue developing post-inoculation is asymptomatic and virus-free (Ratcliff et al., 1997). Furthermore, silencing of viral RNA is active in the recovered tissue, conferring effective resistance to superinfection by W22 and a closely related strain, but not by unrelated strains. In a second example, recovery from infection by cauliflower mosaic virus (CaMV) was studied in kohlrabi *(Brassica oleracea* var. *gongyloides)* (Covey et al., 1997) and oilseed rape *(Brassica napus)* (Al-Kaff et al., 1998). Again, recovery was closely associated with the specific loss of viral

RNA independent of the presence of homologous transgenes. (In each case, the process was also monitored by using viral sequences present in the plants in transgenic form, but transgenic sequences were not essential for the induction of recovery).

The examples of virus resistance mentioned above involve very diverse viruses: TBRV is a single-stranded RNA-virus, while CaMV is an unrelated, double-stranded DNA virus. Therefore, the discovery that these natural viral defense systems resemble gene silencing is highly significant as it indicates that gene silencing may be a general antiviral response in plants. An important unresolved issue, however, is the extent to which viruses, other than those mentioned, elicit silencing upon infection. Most compatible virus-host combinations do not show the recovery phenotypes as do TBRV and CaMV. It is possible, however, that silencing is triggered transiently to limit the extent of virus replication in infected tissue, thus lessening the extent of disease, but in time to limit systemic spread of the virus. Therefore, silencing, as a general antiviral response in both compatible and incompatible virus-host combinations, deserves careful attention.

Viruses fight back – suppression of gene silencing in virus-infected plants

Although plants may combat virus infections by gene silencing, there is recent evidence that some plant viruses can fight back by suppressing the plant's ability to carry out the silencing process. The first indications of this come from studies of viral synergy, where joint infection with two viruses leads to a strong enhancement of symptoms (Matthews et al., 1991). For example, infection of plants with various potyviruses increases the virulence of several unrelated viruses. The agent that causes this synergy is a potyviral protein, the so-called helper component proteinase (HC-Pro). This multifunctional (aphid transmission factor and proteinase) viral protein has been known for its ability to enhance the infection capability of other viruses. It has now been shown that the sequence of the potyvirus TEV that codes for HC-Pro – with the adjacent sequence encoding the P1 viral protein – is sufficient to promote a synergistic infection with three other unrelated viruses, PVX, cucumber mosaic virus (CMV) and TMV (Pruss et al., 1997).

How does this promotion of viral infection work? Three recent reports have revealed that HC-Pro of the potyviruses TEV (Ananthalakshmi et al., 1998, Kasschau and Carrington, 1998) or potato virus Y (Brigneti et al., 1998) makes plants more susceptible to other viruses by suppressing their capacity to carry out PTGS. The studies used transgenic tobacco lines containing reporter genes (reporters of gene silencing) that encode either GUS or GFP. The constructs were designed such that, when silencing occurred, the reporter genes were off, but if it was reversed, they came back on. The general conclusion of all three studies was that expression of HC-Pro, either in virus-infected plants or in stably transformed plant lines causes a general suppression of silencing. How this is achieved is not clear, but the restoration of transgene (GUS and GFP) expression in leaves that were previously silenced for GUS or GFP suggests that the maintenance stage of silencing is blocked.

CMV, a plant virus unrelated to potyviruses is also known to exhibit suppression of PTGS. Infection of tobacco or *Arabidopsis* plants with this virus can suppress PTGS in newly developing leaves (Bèclin et al., 1998), and Brigneti et al. (1998) have shown that such suppression depends on the 2b protein of CMV. Furthermore, the 2b protein of a related cucumovirus, tomato aspermy virus (TAV) was also identified as a virulence determinant and suppressor of PTGS (Li et al., 1999). Significantly, the 2b protein was already known to be a general suppressor of host defense responses against viruses. Unlike the suppression of silencing by HC-Pro, however, suppression by the 2b protein was effective only in tissues newly emerging from the apical meristem. Thus, the 2b protein might prevent the initiation of new silencing or block transport of the silencing signal (as opposed to the inhibition of PTGS-maintenance).

Not only it has been discovered that plants can mount a specific "immune" response to the foreign nucleic acids of viruses – gene silencing – but also that viruses, in turn, have evolved their own counter defense – suppression of silencing. However, we still do not fully understand how viruses suppress plant defense responses that involve gene silencing.

Concluding remarks

In light of recent research it now seems likely that PTGS and RNA-mediated virus resistance are variations of the same phenomenon (see van den Boogaart et al., 1998, Smyth, 1999). However, the mechanism actually responsible for PTGS is still unknown. The two currently accepted models that attempt to explain the mechanism(s) of PTGS (the threshold level and aberrant mRNA models) have not been fully proven so far. Future research on PTGS should look for the following:

– the role for plant-encoded RdRPs in the production of antisense RNAs during PTGS.

– the role of transgene structure in the production of aberrant mRNAs.

– identification and characterization of the proposed aberrant mRNAs and clarification of their function in PTGS.

– identification of genes and their products involved in PTGS.

The use of PTGS for creating virus-resistant plants (RNA-mediated virus resistance) has advantages and disadvantages. The resistance is not easily overcome and does not suffer from the presence of foreign proteins. However, the high sequence specificity makes it questionable whether the resistance is durable, due to selection of resistance breaking strains. Also, the resistance spectrum is rather narrow. The use of multiple transgene constructs that contain sequences derived from different viruses may solve this latter problem but could also create complications associated with transcriptional silencing, because of homologous sequences in transformation vectors (Prins and Goldbach, 1996, van den Boogart et al., 1998). On the other hand, the observation that linking virus-derived sequences to nontarget sequences can reduce the necessary length of homologous transgene to induce resistance suggests that linking several viral sequences together could be an effective way of inducing RNA-mediated virus resistance with a broad spectrum (Pang et al., 1997).

If virus resistance based on PTGS is to be commercially exploited, one should be aware of the potential complications caused by the developmental dependence of PTGS. This means that one cannot be sure if plants are resistant at any given time of their life cycle. In addition, Meyer et al. (1992) have shown that in the case of transgenic petunias there is significantly more variation in gene silencing under field conditions than in the greenhouse. These traits should be further investigated during field trials.

Overall, it seems that PTGS is a useful phenomenon to combat viral infections. Therefore, it should not be surprising that PTGS is not restricted to transgenic plants but similar mechanisms also exist in nature.

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