Minireview

A conserved mechanism for post-transcriptional gene silencing?

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Abstract

Proteins with homology to RNA-directed RNA polymerases function in post-transcriptional gene silencing: in quelling in the fungus *Neurospora crassa*, RNAi in the nematode *Caenorhabditis elegans*, and co-suppression in the mustard plant *Arabidopsis thaliana*. These findings are consistent with a conserved mechanism operating in these diverse species.

Post-transcriptional gene silencing (PTGS) is a general term for a variety of phenomena that repress gene expression by causing degradation of mRNA. PTGS was discovered 'by accident' in organisms that carried a transgene, were virally infected, or were treated with exogenous RNA. A form of PTGS triggered by transgenic DNA, called co-suppression, was initially described in plants [1,2], and a related phenomenon, termed quelling, was later observed in the filamentous fungus *Neurospora crassa* [3]. Co-suppression was first noticed when a transgenic petunia, expected to express a transgene involved in pigment formation at a high level, instead expressed neither the transgene nor related, endogenous genes [1,2]. Subsequent work indicated that viral infection can also trigger co-suppression in plants [4], leading to the hypothesis that the biological role of PTGS is as an anti-viral defense mechanism. Meanwhile, other experiments with the soil nematode *Caenorhabditis elegans* uncovered a phenomenon triggered by double stranded (ds) RNA, called RNA interference (RNAi), as well as transgene-induced co-suppression [5,6]. RNAi is an extremely valuable tool for 'reverse' genetic studies because it gives researchers a quick means to determine the loss-of-function phenotype for a gene. A wide variety of organisms have now been shown to respond to RNAi, including *Drosophila* and mouse (see, for example, [7-9]).

Because of the utility of PTGS for reverse genetic studies and its possible clinical uses, there is a great deal of interest in the underlying molecular mechanism(s). It has become apparent that different PTGS phenomena have common characteristics, and this realization has led to the speculation that PTGS in different organisms may be mediated by similar molecular mechanisms [6,10,11]. Recent genetic and molecular studies provide additional support for this hypothesis. Genes involved in PTGS have been identified in *N. crassa* (*qde* genes) [12], *C. elegans* (*rde*, *mut*, and *ego-1* genes) [13-15], and the mustard plant *Arabidopsis thaliana* (*sde*, *sgs* genes) [16-18]. Intriguingly, related proteins function in PTGS in these organisms (Table 1), as would be expected if different forms of PTGS occur by similar mechanisms.

Among proteins associated with PTGS to date, the most widely conserved are those with homology to tomato RNA-directed RNA polymerase (RdRP) [19]: *Neurospora* QDE-1 [20], *C. elegans* EGO-1 [15], and *Arabidopsis* SGS-2/SDE-1 ([17,18]; Figure 1). The recent addition of *Arabidopsis* genes to this collection suggests that PTGS is a widely conserved, evolutionarily ancient means of gene regulation. Two additional protein families have been linked to PTGS in nematodes and *Neurospora* (Table 1) [13,14,21,22] but such a link has yet to be made in plants. The *rde-1* and *qde-2* genes are members of the *piwi/sting* family. Piwi/Sting proteins are related to eIF2C, a proposed translation factor (see [13,21]); several members of this family are known to have important developmental functions. The *mut-7* and *qde-3* genes encode members of the WRN (Werner's syndrome) protein family, whose members include several RecQ DNA helicases; Werner's syndrome is

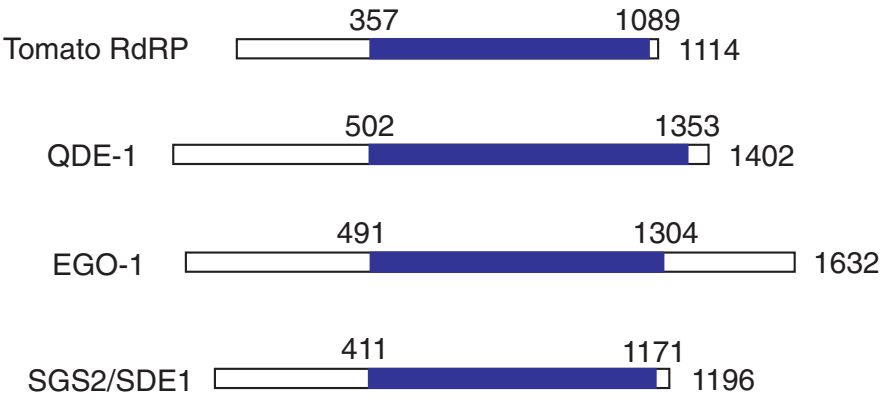

Figure 1
Schematic alignment of tomato RdRP with proteins known to be involved in PTGS in fungus (QDE-1), nematodes (EGO-1), and plants (SGS2/SDE1). The region that is conserved among all four proteins is shaded. The total number of amino acids in each protein is listed to the right; amino acid positions delimiting the conserved regions are indicated. Within the shaded region, the percentage of RdRP amino-acid sequence identical to each related gene sequence is as follows: QDE-1, 17%; EGO-1, 25%; SDS2/SDE1, 41%. Sequence blocks of higher identity are scattered within the shaded region. Substantial sequence similarity between RdRP and SGS2/SDE1 is present in the non-shaded region as well; QDE-1 is relatively divergent from the other three proteins.

Table 1

Conserved proteins involved in PTGS

Protein family	*N. crassa*	*C. elegans*	*A. thaliana*
RdRP	QDE-1	EGO-1	SGS2/SDE1
Piwi/Sting	QDE-2	RDE-1	?
WRN protein	QDE-3	MUT-7	?

Gene names in full: EGO, enhancer of *glp-1*; MUT, mutator; QDE, quelling defective; RDE, RNAi defective; RdRP, RNA-directed RNA polymerase; SDE, silencing defective; SGS, suppressor of gene silencing; WRN, Werner's syndrome.

associated with premature aging. QDE-3 and MUT-7 are predicted to have nucleic acid binding activity: QDE-3 has strong homology to the DNA helicase domains [22] and MUT-7 has homology to the RNase catalytic domains [14].

Arabidopsis RdRP-related genes

Independent screens by the Vaucheret and Baulcombe laboratories for co-suppression-defective mutants in *Arabidopsis* have recovered mutations in a RdRP-related gene, named *sgs2* (suppressor of gene silencing) [17] or *sde1* (silencing defective) [18]. For the purposes of this review, the gene is referred to as *sgs2/sde1*. Mutants were isolated by screening for individuals defective in transgene-induced PTGS. Several other RdRP-related genes exist in *Arabidopsis*, but no mutations were recovered in those genes.

Because co-suppression in plants can be triggered by viral infection and, in fact, is hypothesized to be a viral defense mechanism, both groups investigated the effects of viral infection on *sgs2/sde1* mutants. Was virally induced PTGS defective? Were the mutants super-susceptible to viral infection? Dalmay and colleagues [18] found that PTGS triggered by tobacco mosaic virus and tobacco rattle virus is not defective in *sde1* mutants. They interpret their data as meaning that SDE1 is not required for virally induced PTGS, but only for transgene-induced PTGS. They suggest that virally encoded RdRP activity can substitute for the putative RdRP activity of SDE1. In contrast, Mourrain and colleagues [17] found that *sgs2* mutants had increased susceptibility to infection by certain viruses, such as a cucumovirus, but not others, such as a tobamovirus or a potyvirus. They interpret these results as indicating that PTGS indeed acts as a defense against at least some viruses. Interpretation of infection data is complicated by the fact that many viruses can inhibit PTGS: tobamovirus and potyvirus strongly inhibit PTGS, whereas cucumovirus inhibits it only weakly [17,23]. Logically, the loss of a plant component of PTGS, such as SGS2/SDE1, will have no effect on a virus that fully blocks PTGS by itself, but would allow a higher level of infection by a virus that disables PTGS only partially or not at all. At any rate, these results suggest that the Dalmay *et al.* model is unlikely to be correct for all viruses; in at least some cases, viral RdRP cannot replace SGS2/SDE1.

The RdRP protein family

RdRP activity has been known in plants for decades, although its physiological function has been unclear [24].

Many models for PTGS in plants postulate an RNA amplification step that could be accomplished by RdRP activity (for example in [25]), but it has yet to be shown definitively that RdRP is in fact involved in PTGS. In light of these models, it is intriguing that proteins related to the purified tomato RdRP have now been linked to PTGS in diverse organisms. It should be noted, however, that these proteins have not yet been shown to have RdRP activity. They contain large regions of sequence conservation with tomato RdRP, which may encode RdRP activity, but they also contain extensive regions of divergent sequences (see Figure 1) [15,17,18].

Analysis of genome sequence data has shown that *C. elegans* and *Arabidopsis* contain several RdRP-related genes. In *C. elegans*, for example, there are three *rrf* (RdRP family) genes in addition to *ego-1*. Thus far, six other RdRP-related genes have been found in the *Arabidopsis* genome in addition to *sgs2/sde1*. Genome sequencing has uncovered RdRP-related genes in many other organisms, including several plant species, such as wheat and petunia, and the fission yeast *Schizosaccharomyces pombe*. It is not yet clear though how many of these RdRP-related proteins actually function in PTGS. Some of them may have other cellular functions, perhaps in RNA metabolism. Indeed, *ego-1* was identified originally as being important in development of the *C. elegans* germ line, and was only later shown to function in RNAi; the connection between development and RNAi is not yet clear, but one possibility is that EGO-1 protein has different functions in the two processes. Since alleles of only one RdRP-related gene, *sgs2/sde1*, were recovered as suppressors of PTGS, perhaps the related genes in *Arabidopsis* do not function in PTGS. Alternatively, some or all of these genes may be redundant, so that simultaneous mutations in two or more of them would have to be induced in order to see a phenotype. In addition, if any RdRP-related gene is essential, it would not have been identified because the screens for silencing-defective mutants did not allow for recovery of lethal mutations.

RdRP-related genes are apparently absent from at least one organism that is susceptible to RNAi: *Drosophila melanogaster*. Genome sequence data are nearly complete for *Drosophila*, yet no gene with homology to either tomato or viral RdRP has been found. Since viral and cellular (for example, tomato) RdRP have little amino acid homology to each other, perhaps RdRP function in *Drosophila* is accomplished by an enzyme with yet a different amino acid sequence. Alternatively, RNAi in *Drosophila* may not require RdRP activity at all.

SGS3

Molecular characterization of another *Arabidopsis sgs* gene, *sgs3*, by Mourrain *et al.* [17] revealed that it encodes a novel protein. In general, *sgs3* mutants behave like *sgs2* mutants, including having an increased susceptibility to cucomovirus infection. Thus, it appears that SGS3 is as critical to PTGS as is SGS2/SDE1. No SGS3 relative has been found yet in any other organism, including those with fully sequenced genomes such as *C. elegans* and *Drosophila* and this result is consistent with SGS3 functioning in a plant-specific aspect of PTGS.

The extensive and growing genome sequence database is an important resource for studying the mechanics of PTGS and the role of RdRP-related genes in this and other cellular processes. Three protein families are now known to be important to PTGS. Genome sequence information has allowed researchers to identify paralogs and orthologs very quickly, and provides a focus for future experiments. For example, reverse genetic approaches can be used to generate deletion mutations in these related genes for use in studying their possible role in PTGS. We can expect a more complete molecular portrait of PTGS to be revealed in the near future**.**

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