Silencing genes silencing genes

Unified theories, so beloved of physicists, help our simple minds explain the complex. Now, the labyrinthine phenomenology of gene silencing in plants, quelling in fungi and RNA interference in animals, is approaching a state of unity after recent landmark publications. Two of these, by leading plant groups in the field – headed by Hervé Vaucheret at INRA (Versailles, France) and David Baulcombe at the Sainsbury Laboratory (John Innes Centre, Norwich, UK) – describe the first characterization of Arabidopsis genes involved in post-transcriptional gene silencing (PTGS). PTGS in plants involves down-regulation of gene expression at the post-transcriptional level, rather than at the transcriptional level, by targeting specific RNAs for degradation. Transgenes are subject to suppression by PTGS, as are other genes that share significant sequence homology with the silenced gene (called cosuppression). Plant PTGS is similar to events discovered in other eukaryotes, including suppression of transgenes and transposons, and cellular responses to double-stranded RNAs (dsRNAs). In plants, questions have focused on the role of PTGS, how it is initiated, and how RNA degradation is regulated. Genetics is now being used to identify plant genes at the heart of this mechanism.

Silencing invaders

Originally, gene silencing in plants seemed only to be associated with the abnormal condition of plant transformation by transgenes. Then, plant viruses were found to be involved, both as initiators and targets of PTGS in transgenic plants. Finally, with the discovery that plant viruses could trigger PTGS during normal infections, it became clear that gene silencing was involved in controlling invasive genes. Transgenes can also be considered as invaders, therefore it is not surprising that organisms have evolved steps to limit potentially mutagenic DNA. Indeed, in the nematode worm Caenorhabditis elegans, transposons can be regulated by a PTGS-like process. Such transposon silencing is also linked with another phenomenon called RNA interference (RNAi). RNAi is initiated by the application of dsRNA to cells, which causes the degradation of homologous cellular RNAs. Some classes of nematode mutants deficient in RNAi sensitivity show enhanced transposon mobilization. Early on, dsRNA was seen as a possible intermediate in plant PTGS. Subsequently, the presence of small ~25 nt sense and antisense RNA products of silenced genes were associated with PTGS (Ref. 7). The mechanisms of RNAi-elicited mRNA degradation have recently been studied in Drosophila cell extracts using exogenously added dsRNA and target mRNA (Ref. 8). Notable amongst the intermediates, are ~25 nt RNAs.

‘Visible’ silence

Molecular biology has revealed many fragmentary pieces of the complex PTGS jigsaw; the power of genetics is now cutting a swathe through the confusion. In the fungus Neurospora, transgene-mediated silencing of carotenoid biosynthetic genes, causing an albino phenotype, has been used to study the process of gene ‘quelling’. Analysis of quelling-defective Neurospora mutants has shown that a gene similar to tomato RNA-dependent RNA polymerase (RdRP) is a central player. Involvement of RdRP in plant PTGS had been postulated to explain how a homology-dependent signal targeting RNA for degradation might be transferred from the triggering RNA to other homologous RNAs. So how do Vaucheret’s and Baulcombe’s groups bring the various observations in plants, fungi and invertebrates together in their two recent papers? The approach taken to isolate the PTGS genes relied upon the use of transgenic Arabidopsis lines containing visible reporter transgene constructs, expressing β-glucuronidase (GUS) and green fluorescent protein (GFP), respectively. The transgenes exhibited PTGS either by selection of a silenced line (GUS) or by induction through crossing a PTGS-triggering Amplicon – an integrated copy of potato virus X carrying a GFP marker gene – from one plant line into a second line containing a non-silenced GFP transgene, causing it to be silenced. The silenced Arabidopsis lines were mutated, and loss of PTGS was scored by observing enhanced reporter gene expression and increased mRNA stability. At least four distinct Arabidopsis loci appear to be necessary for PTGS (Ref. 2). Isolation and sequencing of some of the mutant loci showed that genes sgs2 (suppressor of gene silencing) and sde1 (silencing defective) were similar to the tomato RNA-dependent RNA polymerase (RdRP) gene, perhaps not unexpected but no less a satisfying and significant discovery. A second mutant gene (sgs3) was also characterized but has no database matches yet.

Silencing genes

The Arabidopsis PTGS mutants exhibit no obvious morphological phenotypes that might suggest a role in plant growth, but the sgs2 and sgs3 plants both show enhanced susceptibility to cucumber mosaic virus (CMV) but not to infection by two other unrelated viruses. Also, the sde1 mutation has little effect on tobacco mosaic virus or tobacco rattle virus infections. Because the mutant plants had been selected by scoring loss of transgene PTGS, not viral PTGS, this suggests that PTGS operates differently on transgenes and viruses. The Baulcombe group argues that RdRP (SDE1 or SGS2) is required for generating a dsRNA as a prelude to targeting RNA for degradation. Because RNA viruses produce their own RdRP and make dsRNA intermediates during replication, they should not require the services of the host RdRP, hence the lack of effect of the sde1 mutation on virus susceptibility. However, the Vaucheret group came to different conclusions because plants containing either of the PTGS mutations (sgs2 or sgs3) both showed increased susceptibility to CMV. These variant responses to viral pathogens might be a reflection of
the ways different viruses counter PTGS. Alternatively, it might suggest that there are multiple entry points for viral RNAs into the PTGS network. Thus, viruses entering the network directly as dsRNA and not requiring the host RdRP would elicit the same pathogenic response in wild type and sde1 or sgs2 plants. Other viruses such as CMV showing enhanced pathogenicity in sde1 or sgs2 plants might require the host RdRP to promote PTGS. An RdRP activity in plants, stimulated by virus infection, has been known for almost 30 years11 but it remains to be seen whether this is related to PTGS or pathogen defence. Because the sgs3 plants are also more susceptible to CMV, perhaps SGS3 is an accessory factor for host RdRPs involved in PTGS.

Silencing unity
The sde1 mutation also affects the accumulation of a ~25 nt RNA, correlated with PTGS. Release of PTGS in sde1 plants caused significant reduction in the transgene-specific ~25 nt RNA species but not the virus-specific ~25 nt RNA. This further supports the view that transgenes and viruses might have different entry points into PTGS pathways. The ~25 nt RNA also provides an important bridge between PTGS, RdRP and RNAi in different organisms. Some predictions can now be made about possible PTGS circuits in plants (Fig. 1). Initiation of PTGS by invasive nucleic acid presumably involves detection of RNA by a surveillance system. If the invader is DNA (transgene, transposon, rearranged gene or DNA virus), the triggering RNA should arrive in the cytoplasm from the nucleus. RNA viruses provide a triggering RNA directly in the cytoplasm. The host RdRP might be involved in the initial recognition event, or later on if the enzyme needs to be activated or synthesized. The host RdRP would convert target RNA into a dsRNA with an alternative route for RNA viruses using viral RdRP. Choice of entry points for viral and transgene RNAs might explain the variant pathologies of plant RdRP mutants to viruses12. In RNAi, a dsRNA enters the process without the requirement for RdRP (Fig. 1). Once PTGS has been triggered, the initiating agent is no longer needed to maintain it, a characteristic of plant PTGS (Ref. 12).

The biochemical steps generating RNA intermediates during degradation of the target RNA have been studied best in vitro with animal cell extracts13 and it remains to be seen what steps are common to plants. In the RNAi scheme, ~25 nt RNAs are products of a ribonuclease (RNase) and helicase acting on dsRNA. A complex of the RNase and the ~25 nt RNA is guided to the homologous target RNA. Degradation of the target RNA also regenerates more ~25 nt RNA. This propagative step probably explains the maintenance of PTGS seen in plants. The autonomy of PTGS would require a continued supply of target RNA, hence the persistence of transcription of nuclear genes subject to PTGS.

This speculative model in which RdRP plays a central role brings together independent observations from different systems. However, the SGS3 locus has no obvious relatedness to genes involved in quelling or RNAi phenomena in other organisms. So what is different about plants? Plant cells communicate locally through plasmodesmata and systemically via vascular tissues. Viruses move in this way, as do diffusible signals of PTGS. Perhaps SGS3 is required for linking mobile PTGS signals to the cellular PTGS machinery1, possibly via a regulatory receptor. Regulating PTGS is important because there must be sufficient flexibility to detect a broad spectrum of invasive nucleic acids while discriminating against endogenous RNAs. We will need both genetics and biochemistry to tell us how this regulation works.

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Metabolic engineering of plant carotenoids

Metabolic engineering of carotenoid biosynthesis has been achieved recently in several plants. β-carotene (provitamin A) levels have been increased in rice and canola seeds2 and in tomato fruits3 and astaxanthin, a non-plant carotenoid, has been produced in tobacco flowers4. The ‘nuts and bolts’ of these first transformation experiments are discussed.

Carotenoids are synthesized de novo from geranylgeranyl diphosphate (Fig. 1) by all photosynthetic organisms, where they participate in light harvesting and photoprotection from excess light energy. Many non-photosynthetic bacteria (Erwinia herbicola, Deinococcus radiodurans, Thermus aquaticus) and fungi (Neurospora crassa, Phycomyces blakesleeanus), also synthesize carotenoids. In plants, carotenoids accumulate together with chlorophyll in leaf chloroplasts, but without chlorophyll in the chromoplasts of many fruits, seeds and flowers. Although the carotenoid composition of chloroplasts is relatively invariant, a wide range of different carotenoids can be found in chromoplasts: lycopene in tomato fruits, β-carotene (pro-vitamin A) in carrot roots, lutein and zeaxanthin in maize endosperm (Fig. 1). The molecular biology of the plant pathway is well elucidated6.

Vertebrates do not synthesize carotenoids. Nevertheless, they depend on dietary carotenoids for making their retinoids, such as retinal (the main visual pigment), retinol (vitamin A), and retinoic acid (a substance controlling morphogenesis; Fig. 1). The main precursor of retinoids is β-carotene, a